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(57) Abstract

The present invention relates to the novel use of organic molecules capable of inhibiting C-proteinase activity in order to regulate, modulate and/or inhibit abnormal collagen formation.

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C-PROTEINASE INHIBITORS FOR THE TREATMENT OF DISORDERS RELATED TO THE OVERPRODUCTION OF COLLAGEN

This application is related to and is a continuation-in-part application of the U.S. application Serial Number 08/601,203, entitled "C-Proteinase Inhibitors For The Treatment Of Disorders Related To The Overproduction Of Collagen," filed February 14, 1996, which is a continuation-in-part application of the provisional U.S. application Serial Number 60/002,038, filed August 8, 1995.

1. FIELD OF THE INVENTION

Collagen is integral to, among other things, the proper formation of connective tissue. Thus, the over- or under-production of collagen or the production of abnormal collagen (including incorrectly processed collagen) has been linked with numerous connective tissue diseases and disorders. Mounting evidence suggests that C-proteinase is an essential key enzyme for the proper maturation of collagen, and therefore appears to be an ideal target for the inhibition, control and/or modulation of collagen formation.

The present invention relates to organic molecules capable of inhibiting C-proteinase activity in order to regulate, modulate and/or inhibit abnormal collagen formation. More specifically, the invention relates to the use of compounds and pharmaceutical compositions thereof for the treatment of various diseases relating to the inappropriate or unregulated production of collagen.

2. BACKGROUND OF THE INVENTION

Collagen Structure. At present nineteen types of collagens have been identified. These collagens, including fibrillar collagen types I, II, III are synthesized as procollagen precursor molecules which contain amino- and

carboxy-terminal peptide extensions. These peptide extensions, referred to as "pro-regions," are designated as N- and C-propeptides, respectively.

The pro-regions are typically cleaved upon secretion of the procollagen triple helical precursor molecule from the cell to yield a mature triple helical collagen molecule. Upon cleavage, the "mature" collagen molecule is capable of association, for example, into highly structured collagen fibers. *See e.g.*, Fessler and Fessler, 1978, *Annu. Rev. Biochem.* 47:129-162; Bornstein and Traub, 1979, *in: The Proteins* (eds. Neurath, H. and Hill, R.H.), Academic Press, New York, pp. 412-632; Kivirikko *et al.*, 1984, *in: Extracellular Matrix Biochemistry* (eds. Piez, K.A. and Reddi, A.H.), Elsevier Science Publishing Co., Inc., New York, pp. 83-118; Prockop and Kivirikko, 1984, *N. Engl. J. Med.* 311:376-383; Kuhn, 1987, *in: Structure and Function of Collagen Types* (eds. Mayne, R. and Burgeson, R.E.), Academic Press, Inc., Orlando, Florida, pp. 1-42.

Diseases Associated With The Abnormal Production of Collagen. An array of critical diseases has been associated with the inappropriate or unregulated production of collagen, including pathological fibrosis or scarring, including endocardial sclerosis, idiopathic interstitial fibrosis, interstitial pulmonary fibrosis, perimuscular fibrosis, Symmers' fibrosis, pericentral fibrosis, hepatitis, dermatofibroma, billary cirrhosis, alcoholic cirrhosis, acute pulmonary fibrosis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, kidney fibrosis/glomerulonephritis, kidney fibrosis/diabetic nephropathy, scleroderma/systemic, scleroderma/local, keloids, hypertrophic scars, severe joint adhesions/arthritis, myelofibrosis, corneal scarring, cystic fibrosis, muscular dystrophy (duchenne's), cardiac fibrosis, muscular fibrosis/retinal separation, esophageal stricture, payronles disease. Further fibrotic disorders may be induced or initiated by surgery, including scar revision/plastic surgeries, glaucoma, cataract fibrosis, corneal scarring, joint adhesions, graft vs. host disease, tendon surgery, nerve entrapment, dupuytren's contracture, OB/GYN adhesions/fibrosis, pelvic adhesions, peridural fibrosis, restenosis. One strategy for the treatment of these diseases

is to inhibit the pathological overproduction of collagen. Thus, identification and isolation of molecules which control, inhibit and/or modulate the production of collagen are of major medical interest.

Relationship Between Collagen Formation and C-Proteinase. Recent evidence suggests that C-proteinase is the essential key enzyme that catalyzes the cleavage of the C-propeptide of, for example, fibrillar collagens, including type I, type II, and type III collagen. *See*, U.S. Application Ser. No. 60/002,038, filed August 8, 1995 as a provisional application, and references disclosed therein.

C-proteinase was first observed in the culture media of human and mouse fibroblasts (Goldberg *et al.*, 1975, *Cell* 4:45-50; Kessler and Goldberg, 1978, *Anal. Biochem.* 86:463-469), and chick tendon fibroblasts (Duskin *et al.*, 1978, *Arch. Biochem. Biophys.* 185:326-332; Leung *et al.*, 1979, *J. Biol. Chem.* 254:224-232). An acidic proteinase which removes the C-terminal propeptides from type I procollagen has also been identified. Davidson *et al.*, 1979, *Eur. J. Biochem.* 100:551.

A partially purified protein having C-proteinase activity was obtained from chick calvaria in 1982. Njieha *et al.*, 1982, *Biochemistry* 23:757-764. In 1985, chicken C-proteinase was isolated, purified and characterized from conditioned media of chick embryo tendons. Hojima *et al.*, 1985, *J. Biol. Chem.* 260:15996-16003. Murine C-proteinase has been subsequently purified from media of cultured mouse fibroblasts. Kessler *et al.*, 1986, *Collagen Relat. Res.* 6:249-266; Kessler and Adar, 1989, *Eur. J. Biochem.* 186:115-121. Finally, the cDNA encoding human C-proteinase has been identified, as set forth in the above-referenced related applications and references disclosed therein.

Experiments conducted with these purified forms of chick and mouse C-proteinase have indicated that the enzyme is instrumental in the formation of functional collagen fibers. Fertala *et al.*, 1994, *J. Biol. Chem.* 269:11584.

C-Proteinase Inhibitors. As a consequence of the enzyme's apparent importance to collagen production, scientists have identified a number of C-

proteinase inhibitors. *See e.g.*, Hojima *et al.*, *supra*. For example, several metal chelators have demonstrated activity as a C-proteinase inhibitor. Likewise, chymostatin and pepstatin A were found to be relatively strong inhibitors of C-proteinase. Additionally, α_2-Macroglobulin, ovostatin, and fetal bovine serum appear to at least partially inhibit C-proteinase activity.

Dithiothreitol, SDS, concanavalin A, Zn^{2+}, Cu^{2+}, and Cd^{2+} are similarily reported to be inhibitory at low concentrations. Likewise, some reducing agents, several amino acids, phosphate, and ammonium sulfate were inhibitory at concentrations of 1-10mM. Further, the enzyme was shown to be inhibited by the basic amino acids lysine and arginine. Leung *et al.*, *supra*; Ryhänen *et al.*, 1982, *Arch. Biochem. Biophys.* 215:230-236. Finally, high concentrations of NaCl or Tris-HCl buffer were found to inhibit C-proteinase's activity. For example, it is reported that, with 0.2, 0.3, and 0.5M NaCl, the activity of C-proteinase was reduced 66, 38, and 25%, respectively, of that observed with the standard assay concentration of 0.15M. Tris-HCl buffer in a concentration of 0.2-0.5M markedly inhibited activity. Hojima *et al.*, *supra*. In contrast, microbial inhibitors such as leupeptin, phosphoramidon, antipain, bestatin, elastinal, and amastatin, are considered to have weak or no effect on the activity of C-proteinase.

C-proteinase activity and its inhibition have been determined using a wide array of assays. *See e.g.*, Kessler and Goldberg, 1978, *Anal. Biochem.* 86:463; Njieha *et al.*, 1982, *Biochemistry* 21:757-764. Despite the availability of such assays, large scale review and testing of potential C-proteinase inhibitors has not been performed to date due to the limited availability of human C-proteinase. As articulated in numerous publications, the enzyme is difficult to isolate by conventional biochemical means and the identity of the cDNA sequence encoding such enzyme was not known until reported in the above-referenced and related patent applications.

Development Of Compounds To Inhibit C-Proteinase Activity. In view of its essential role in the formation and maturation of collagen, C-proteinase appears to be an ideal target for the treatment of disorders

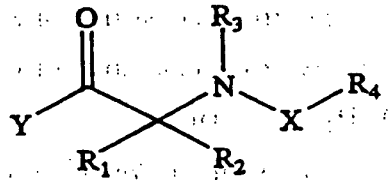
associated with the inappropriate or unregulated production and maturation of collagen. However, none of the inhibitors so far identified has proven an effective therapeutic for the treatment of collagen-related diseases or even an inhibitor to C-proteinase activity.

The identification of effective compounds which specifically inhibit the activity of C-proteinase to regulate and modulate abnormal or inappropriate collagen production is therefore desirable and the object of this invention.

3. SUMMARY OF THE INVENTION

The present invention relates to organic molecules capable of modulating, regulating and/or inhibiting production and/or maturation of collagen by affecting C-proteinase activity. Specifically, the compounds of the present invention have the formulae:

a. ***Inhibitor A***



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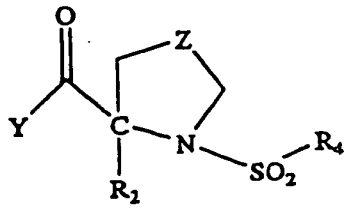


wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of aryl, heteroaryl, alkyl, aralkyl, heteroaralkyl, alkylamino, arylalkylamino;

X is selected from the group consisting of SO_2, C=O;

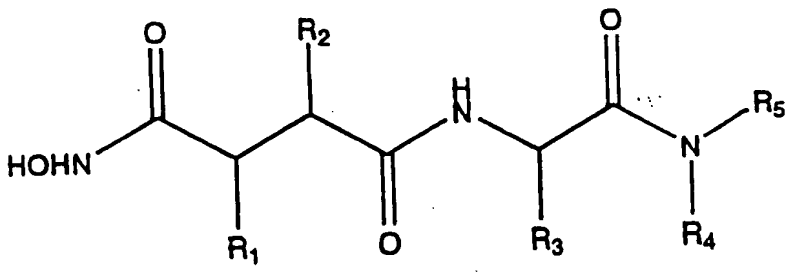
Y is selected from the group consisting of OH, HOHN(hydroxylamine), H_2N, alkylamino;

Z is a direct bond; methylene, oxygen, sulfor, amino;

n is 0 or 1;

or:

b. Inhibitor B



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

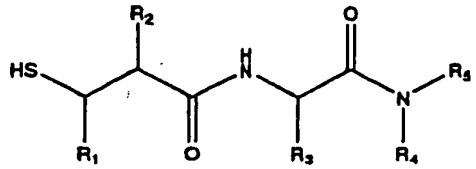
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

c. Inhibitor C



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

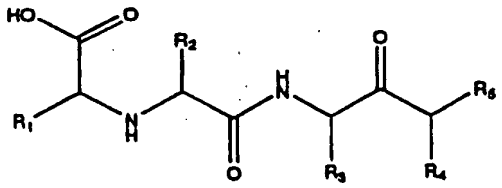
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

d. Inhibitor D



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

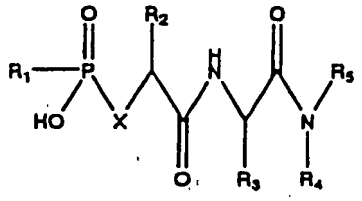
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

e. Inhibitor E



wherein:

R_1 is selected from the group consisting of OH, alkoxyl, lower alkyl, alkylamino, peptide;

X is selected from the group consisting of N, C;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

The present invention is further directed to pharmaceutical compositions comprising a pharmaceutically effective amount of the above-described compounds and a pharmaceutically acceptable carrier or excipient. Such a composition should modulate the production and/or maturation of collagen by inhibiting C-proteinase activity.

The present invention is also directed to the use of the disclosed compounds and compositions for the treatment of disorders associated with the inappropriate or unregulated production of collagen by modulating, inhibiting and/or regulating C-proteinase activity.

More particularly, the compositions of the present invention may be included in methods for treating diseases associated with inappropriate or unregulated production of collagen, including, but not limited to, rheumatoid arthritis, scleroderma, pathological fibrosis or scarring.

4. DEFINITIONS

"C-proteinase" shall be construed to mean an enzyme capable of processing collagen molecules, derivatives or fragments, or their precursors by cleaving through -Ala↓Asp-Asp- and/or -Gly↓Asp-Glu-. The term shall include human C-proteinase and derivatives, analogs, fragments and variants thereof having C-proteinase-like activity.

"Pharmaceutically acceptable salts" refers to those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

"Alkyl" refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. Typical alkyl groups includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tertiary butyl, pentyl, hexyl

and the like. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, carboxyl, hydroxyl, mercapto, cycloalkyl, heterocycloalkyl, halo, alkoxyl, alkylamino.

"Aryl" refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocylic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. Preferably, the aryl is a substituted or unsubstituted phenyl or pyridyl. Preferred aryl substituent(s) preferably phenyl or pydridyl are halogen, trihalomethyl, hydroxyl, SH, NO_2, amine, thioether, cyano, alkoxy, and groups.

5. DETAILED DESCRIPTION OF THE INVENTION

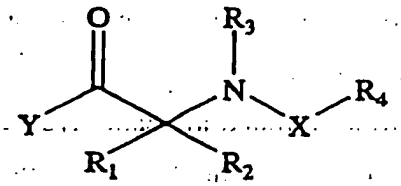
The present invention relates to compounds capable of regulating and/or modulating collagen formation by inhibiting C-proteinase activity.

More particularly, the present invention is directed to compounds which inhibit C-proteinase activity as a therapeutic approach to cure or manage various connective tissue disorders, including fibrotic disorders, arthritic disorders, or disorders induced or initiated by surgical or dramatic insults.

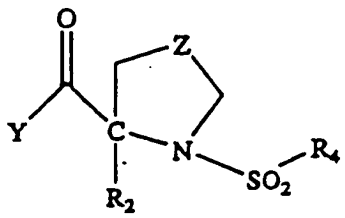
5.1. The Compounds

The invention is generally directed to compounds and/or compositions comprising compounds having the formulae:

a. ***Inhibitor A***



or:



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of aryl, heteroaryl, alkyl, aralkyl, heteroaralkyl, alkylamino, arylalkylamino;

X is selected from the group consisting of SO_2, C=O;

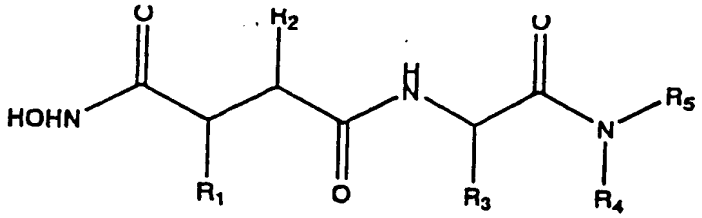
Y is selected from the group consisting of OH, HOHN(hydroxylamine), H_2N, alkylamino;

Z is a direct bond; methylene, oxygen, sulfor, amino;

n is 0 or 1;

or:

b. Inhibitor B



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

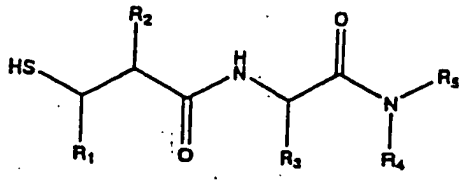
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

c. Inhibitor C



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

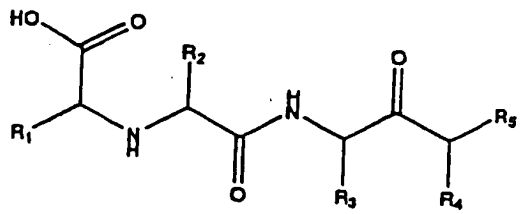
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono-, or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

d. Inhibitor D



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

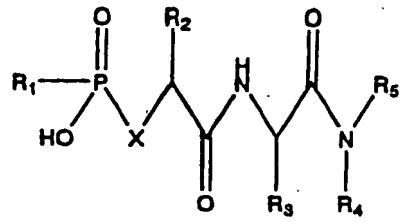
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

or:

e. Inhibitor E



wherein:

R_1 is selected from the group consisting of OH, alkoxyl, lower alkyl, alkylamino, peptide;

X is selected from the group consisting of N, C;

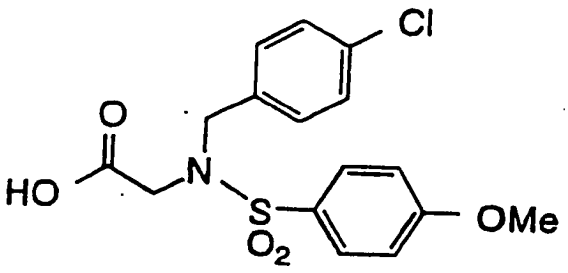
R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl;

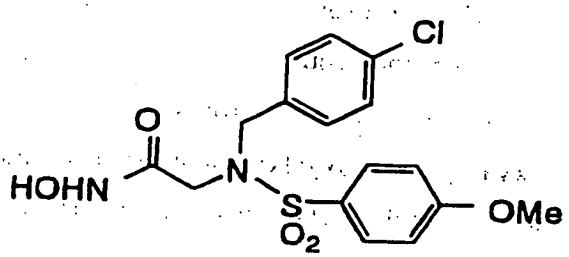
R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

In specific embodiments of the invention, the compounds of the present invention may have the following formulae:



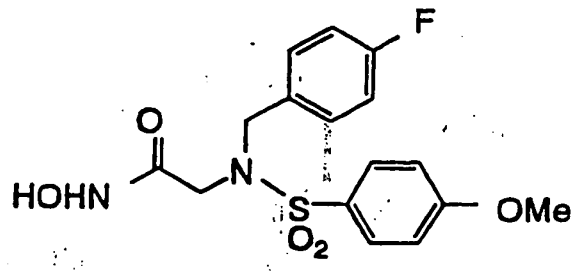
and pharmaceutically acceptable salts thereof;

or:



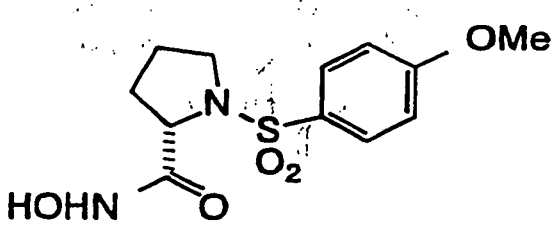
and pharmaceutically acceptable salts thereof;

or:



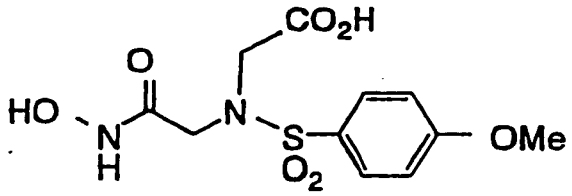
and pharmaceutically acceptable salts thereof;

or:



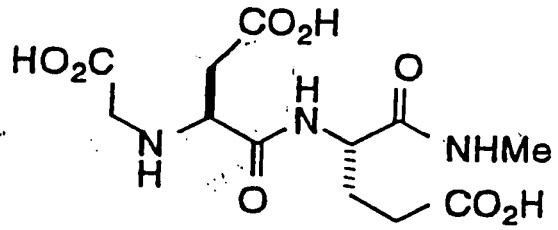
and pharmaceutically acceptable salts thereof;

or:



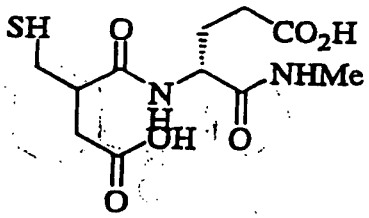
and pharmaceutically acceptable salts thereof;

or:



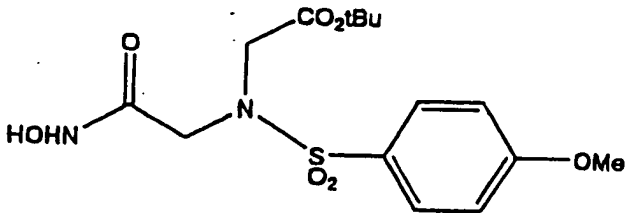
and pharmaceutically acceptable salts thereof;

or:



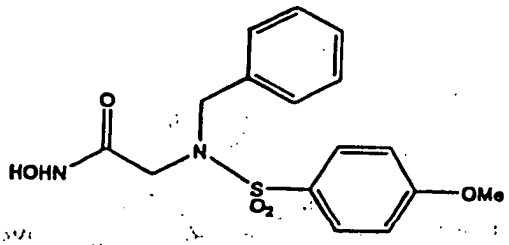
and pharmaceutically acceptable salts thereof;

or:



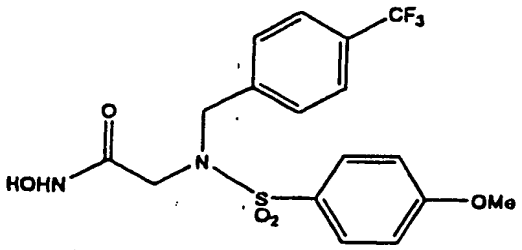
and pharmaceutically acceptable salts thereof;

or:



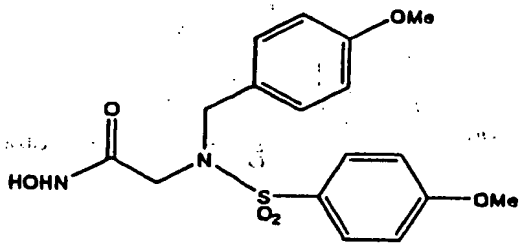
and pharmaceutically acceptable salts thereof;

or:



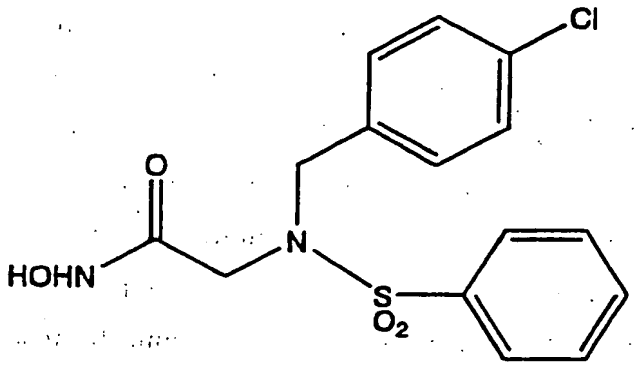
and pharmaceutically acceptable salts thereof;

or:



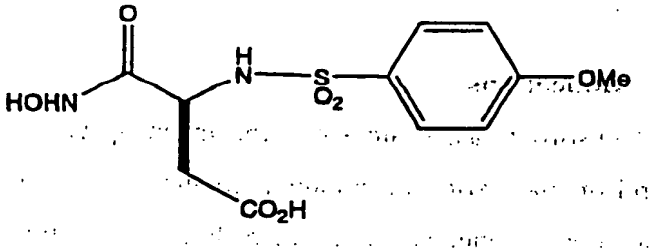
and pharmaceutically acceptable salts thereof;

or:



and pharmaceutically acceptable salts thereof;

or:



and pharmaceutically acceptable salts thereof.

The chemical formulae referred herein may exhibit the phenomenon of tautomerism. As the formulae drawing within this specification can only represent one of the possible tautomeric forms, it should be understood that the invention encompasses any tautomeric form which possesses the ability to

regulate and/or modulate the production and/or maturation of collagen by inhibiting C-proteinase activity. Further, the invention is understood to include all possible stereoisomers of each of the compounds described.

In addition to the above compounds and their pharmaceutically acceptable salts, the invention is further directed, where applicable, to solvated as well as unsolvated forms of the compounds (*e.g.* hydrated forms) having the ability to inhibit, regulate and/or modulate the production and/or maturation of collagen by inhibiting C-proteinase activity.

The compounds described above may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Suitable processes are illustrated by the following representative examples. Necessary starting materials may be obtained by standard procedures of organic chemistry.

An individual compound's relevant activity and efficiency as an agent to affect C-proteinase activity may be determined using available techniques. Preferentially, a compound is subjected to a series of screens to determine the compound's ability to modulate, regulate and/or inhibit the production and maturation of collagen. These screens include biochemical assays, cell culture assays and animal models.

5.2. Indications

Disorders associated with the inappropriate or unregulated production and/or maturation of collagen, including arthritic disorders, fibrotic disorders, and other connective tissue disorders, can be treated with the compounds and compositions of the present invention.

These diseases or disorders include pathological fibrosis or scarring, such as endocardial sclerosis, idiopathic interstitial fibrosis, interstitial pulmonary fibrosis, perimuscular fibrosis, Symmers' fibrosis, pericentral fibrosis, hepatitis, dermatofibroma, billary cirrhosis, alcoholic cirrhosis, acute pulmonary fibrosis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, kidney fibrosis/glomerulonephritis, kidney fibrosis/diabetic

nephropathy, scleroderma/systemic, scleroderma/local, keloids, hypertrophic scars, severe joint adhesions/arthritis, myelofibrosis, corneal scarring, cystic fibrosis, muscular dystrophy (duchenne's), cardiac fibrosis, muscular fibrosis/retinal separation, esophageal stricture, payronles disease. Further, fibrotic disorders may be induced or initiated by surgery such as scar revision/plastic surgeries, glaucoma, cataract fibrosis, corneal scarring, joint adhesions, graft vs. host disease, tendon surgery, nerve entrapment, dupuytren's contracture, OB/GYN adhesions/fibrosis, pelvic adhesions, peridural fibrosis, restenosis. Still further fibrotic disorders may be induced by chemotherapy, including, for example lung fibrosis and the like.

5.3. Pharmaceutical Formulations And Routes Of Administration

The identified compounds can be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms. Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

5.3.1. Routes Of Administration.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as

complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

5.3.2. Composition/Formulation.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or

dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve

to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the

compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days.

Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the C-proteinase inhibiting compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

5.3.3. Effective Dosage.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture (*i.e.*, the concentration of the test compound which achieves a half-

maximal inhibition of the C-proteinase activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD_{50} and ED_{50}. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. *See, e.g.*, Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1.

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the C-proteinase inhibiting effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; for example, the concentration necessary to achieve 50-90% inhibition of the C-proteinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen which maintains plasma

levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%.

In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

5.3.4. Packaging

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of arthritis or any other fibrotic disorder.

6. EXAMPLES

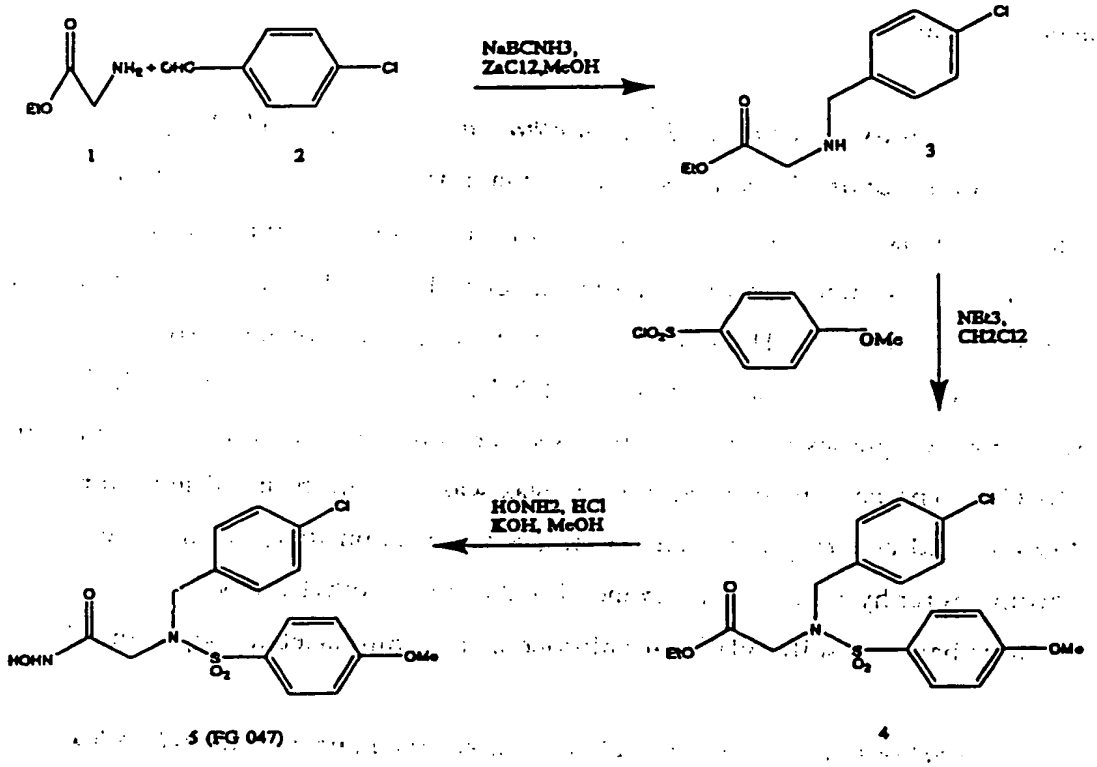
The compounds of the present invention may be synthesized according to known techniques. The following represent preferred methods for synthesizing and testing the compounds of the claimed invention:

6.1. Example 1: Compound Synthesis

6.1.1. Synthesis Of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-chlorobenzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'(4-chlorobenzyl)]amino]-acetamide (5)

(specific compounds in this specification may be referred to, for nomenclature purposes as "C#", wherein "#" is an arabic number), also designated as FG047, (Example for Inhibitor A) is as follows:



Synthesis Of Ethyl-2[(4-chlorobenzyl)amino]-acetate (3). To a cold solution of glycine ethyl ester hydrochloride (1) (1.00 g, 7.16mmol) and 4-chlorobenzaldehyde (2) (1.00 g, 7.16 mmol) in anhydrous MeOH (10 ml) was added anhydrous $ZnCl_2$ solid (75 mg, 0.55 mmol) followed by $NaBCNH_3$ solid (0.45 g, 7.16 mmol). After stirring at room temperature for 18 h, the reaction mixture was quenched with 1 N HCl (20 ml) aqueous solution, and stirred for another 30 min. The mixture was concentrated on a rotary evaporator to remove most MeOH solvent and then extracted with ether (20 ml). The aqueous layer was carefully basified in an ice bath with 45%(w/w) of KOH aqueous solution to pH 10, and extracted with EtOAc (2 x 50 ml).

The combined EtOAc organic layers was washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by silica gel chromatography (4/1 hexanes/EtOAc) to get ethyl-2[(4-chlorobenzyl)amino]-acetate (3) as an oil.

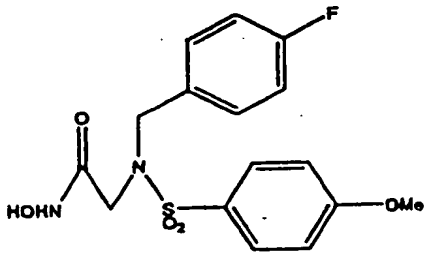
Synthesis Of Ethyl-2-[[N-(4-methoxybenzene-sulfonyl)-N-(4-chlorobenzyl)]amino]acetate (4). Triethylamine (294 mg, 2.90 mmol) was added dropwise to a solution of ethyl-2[(4-chlorobenzyl)amino]-acetate (3) (600 mg, 2.64 mmol) and p-methoxybenzenesulfonyl chloride (545 mg, 2.64 mmol) in anhydrous CH_2Cl_2 (7 ml). The mixture was stirred at room temperature for 15 h and treated with 1 N HCl (20 ml) solution. The resulting two phases were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel chromatography (3/1 to 2/1 hexanes/EtOAc) to get ethyl-2-[[N-(4-methoxybenzene-sulfonyl)-N-(4-chlorobenzyl)]-amino]acetate (4) as an oil.

Synthesis Of N-hydroxy-2[[N'-(4-methoxy-benzenesulfonyl)-N'(4-chlorobenzyl)]amino]-acetamide (5). Separate solutions of hydroxylamine hydrochloride (171 mg, 2.46 mmol) in MeOH (1.3 ml) and of KOH (207 mg, 3.69 mmol) in MeOH (1.3 ml) were prepared at the boiling point, cooled to 40°C, and then the latter solution was added to the former. After cooling the reaction mixture in an ice bath for 30 min, potassium chloride solid was filtered off. To the filtrate was added ethyl ester (4) (487 mg, 1.23 mmol). After stirring at room temperature for 6 h, the reaction mixture was treated with 1 N HCl (20 ml) solution, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to get the crude product. The residue was triturated in ether and the white solid was collected to get the hydroxamate, *i.e.*, N-hydroxy-2[[N'-(4-methoxy-benzenesulfonyl)-N'(4-chlorobenzyl)]-amino]-acetamide (5), also designated as FG-047.

mp: 124 - 125°C; MS (ES) $(M+H)^+$: 385; 1H NMR (360 MHz, DMSO-d6)δ 10.47 (s, 1 H, OH), 8.81 (s, 1 H, NH). 7.81 - 7.08 (m, 8 H, Ph), 4.34 (s, 2 H, CH_2CO), 3.85 (s, 3 H, OMe), 3.63 (s, 2 H, CH_2Ph).

6.1.2. Synthesis Of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-fluorobenzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-fluorobenzyl)]amino]-acetamide (6), also designated as FG053, (Example for Inhibitor A) is essentially as described for the synthesis of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'(4-chlorobenzyl)]amino]-acetamide, using as starting material 4-fluorobenzaldehyde instead of 4-chlorobenzaldehyde. *See*, Section 6.1.1.

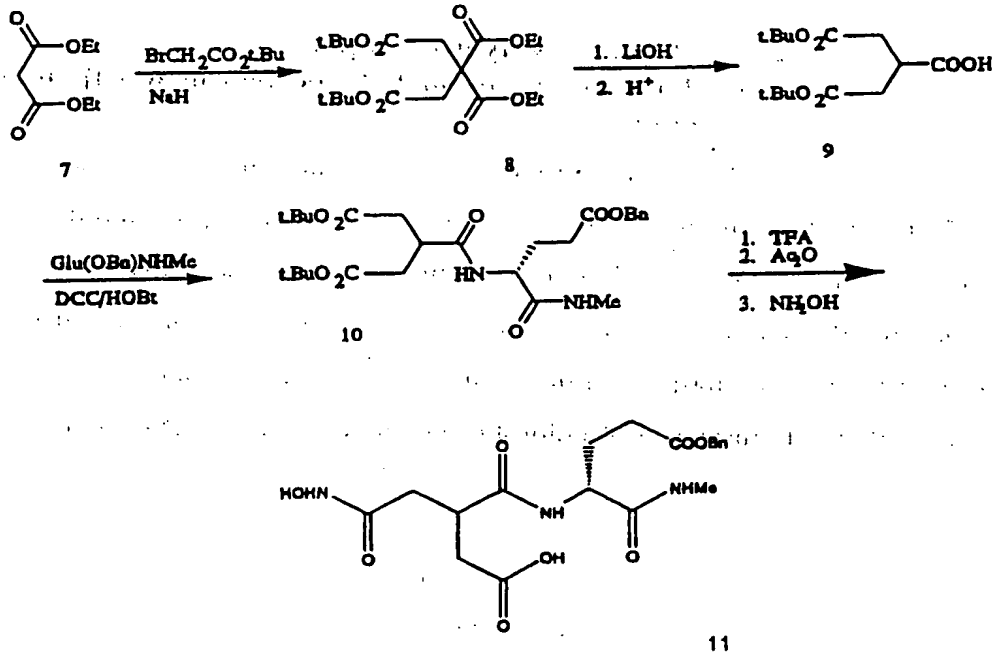


6 (FG 053)

MS (ES) $(M-1)^-$:367; ^1H NMR (360 MHz, DMSO-d6) δ 10.45 (s, 1 H, OH), 8.83 (s, 1 H, NH).7.81-7.08 (m, 8 H, Ph), 4.33 (s, 2 H, CH_2CO), 3.85 (s, 3 H, OMe), 3.62 (s, 2 H, CH_2Ph).

6.1.3. Synthesis Of Hydroxamate

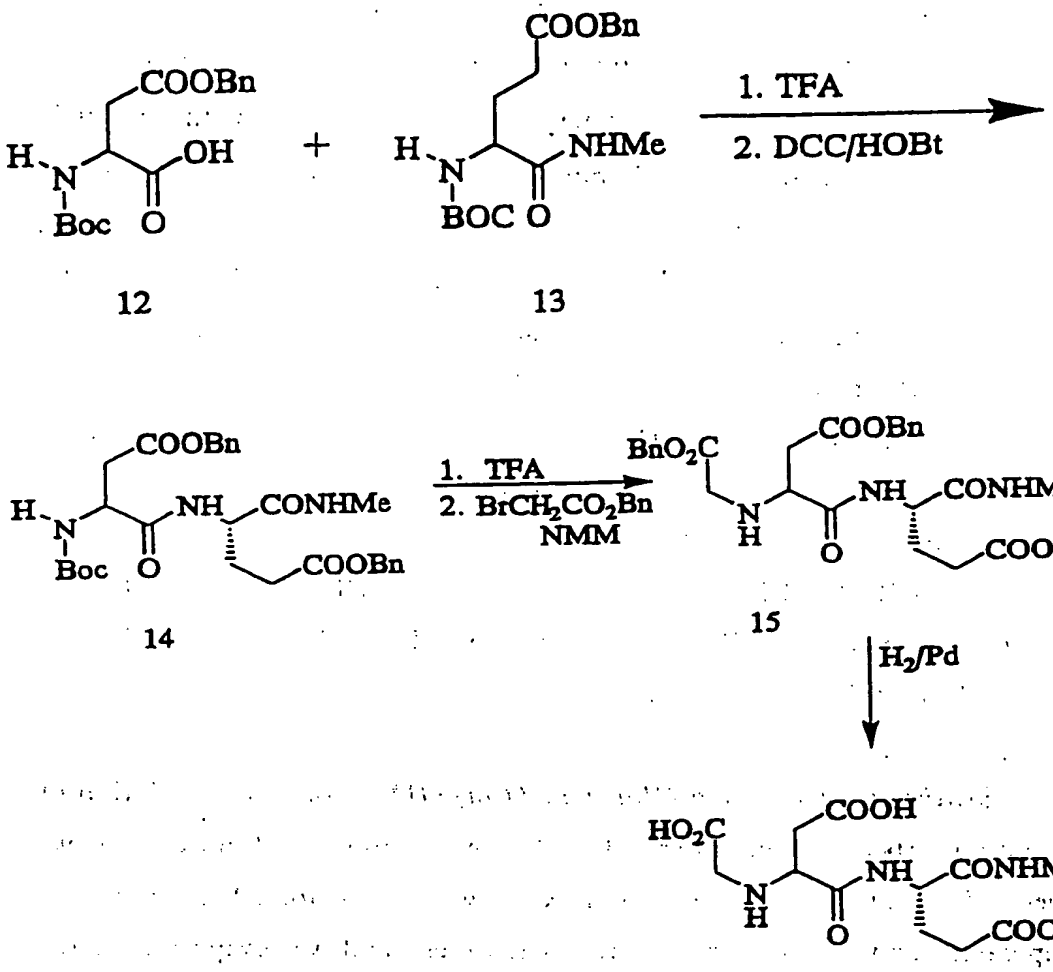
The preferred method for synthesizing hydroxamate (11) (Example for Inhibitor B) is as follows:



Alkylation of malonic ester (7) with tert-butyl bromoacetate provides tetraester (8). Upon saponification and decarboxylation, the resulting aid (9) is coupled with Glu (OBn) NHMe using peptide coupling reaction to give (10). After subsequent hydrolysis, anhydride formation, and addition of NH_2OH, (10) can be converted to the desired hydroxamate (11).

6.1.4. Synthesis Of N-Carboxymethyl Dipeptide

The preferred method for synthesizing N-carboxymethyl dipeptide (16), also designated as FG057, (Example for Inhibitor D) is as follows:



The building blocks (12) and (13) which are readily available by literature procedures, are coupled by the classical DCC/HOBt method leading to the dipeptide (14) in 78% yield. Deprotection of (14) using TFA in CH_2Cl_2 and subsequent alkylation with benzyl bromacetate in the presence of NMM resulted in the formation of the N-carboxymethyl dipeptide (15), which was converted to the free acid (16) by catalytic hydrogenation.

Boc-Glu (OBn)OH and Boc-Asp(OBn)OH have been synthesized using methods readily known in the art.

Synthesis Of Boc-Glu(OBn)NHMe (13): Triethylamine (2.0 g, 20 mmol) was added to a solution of Boc-Glu (OBn)OH (6.7 g, 20 mmol) in dry THF (100 ml). The solution was cooled to -78°C under argon atmosphere, then ethyl chloroformate (2.2 g, 20mmol) was added dropwise. The reaction mixture was allowed to warm to -30°C over 2h, then a 40% aquoeus solution of methyl amine (22 mmol) was add, and the reaction mixture was allowed to warm to room temperature. After the reaction had stirred for a further hour, diethyl ether (50 ml) and water (70 ml) were added. The organic layer was separated, washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution, and dried over $MgSO_4$. The solvent was evaporated in vacuo to give a white solid (5.0 g, 72% yield).

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.38 (s, 9H, CH_3); 1.85 - 2.59 (m, 4H, CH_2); 2.75 (d, 3H, NCH_3); 4.17 (m, 1H, CH); 5.08 (δ, 2H, OCH_2); 5.33 (bδ, 1H, NH); 6.30 (bs, 1H, NH); 7.31 (s, 5H, Ph-H).

Synthesis Of Boc-Asp(OBn)-Glu(OBn)NHMe (14): To a solution of tert-butoxycarbonyl amino ester (13) (700 mg, 2 mmol) in 10 ml CH_2Cl_2 was added 1.5 ml TFA and the reaction mixture was stirred for 1 h at room temperature under argon atmosphere. The excess acid was evaporated under vacuum, the residue was treated several times with diethyl ether and concentrated under reduced pressure, to give a colourless oil, which was without further purification. The TFA-salt, Boc-Asp(OBn)OH (446 mg, 2 mmol), HOBt (170 mg, 2 mmol) and NMM (202 mg, 2 mmol) in CH_2Cl_2 (10 ml) was added and the reaction was stirred overnight at room temperature under argon atmosphere. The precipitate of dicyclohexylurea was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (30 ml), filtrated again and washed with 1 M $NaHCO_2$, 10% citric acid, and saturated NaCl solution. The organic layer was dried over $MgSO_4$, and concentrated in vacuo, to give a white solid in 78% yield (867 mg).

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.41 (s, 9H, CH_3); 1.85 - 3.05 (m, 9H, $3CH_2$, NCH_3); 4.40 (m, 2H, CH); 5.01 (m, 4H, OCH_2); 5.53 (d, 1H, NH); 6.48 (bs, 1H, NH); 7.15 - 7.38 (m, 10H, Ph-H).

Synthesis Of N-Carboxymethyl-Dipeptide (15): To a solution of Boc-Asp(OBn)-Glu(OBn)NHMe (14) (555 mg, 1 mmol) in 10 ml CH_2Cl_2 was added 1 ml TFA and the reaction mixture was stirred for 1 h at room temperature under argon atmosphere. The excess acid was evaporated under vacuum, the residue was treated several times with diethyl ether and concentrated under reduced pressure, to give a colourless oil, which was dissolved in dry THF (20 ml). To this solution was added NMM (101 mg, 1 mmol) and benzyl bromoacetate (230 mg, 1 mmol), and the reaction was stirred overnight at room temperature under argon atmosphere. The reaction mixture was concentrated under reduced pressure. The residue was diluted with EtOAc (20 ml), and washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution. The organic layer was dried over $MgSO_2$, and then concentrated to give a colourless oil, which was purified by flash chromatography on silica gel (ethyl acetate/MeOH 10:1) to afford (15) (410 mg) as a white solid in 88% yield.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.83 - 2.95 (m, 9H, $3CH_2$, NCH_3); 3.30 - 3.60 (m, 4H, CH & N CH_2); 4.39 (m, 2H, CH); 5.05 (m, 6H, OCH_2); 6.64 (bs, 1H, NH); 7.29 - (m, 15H, Ph-H). 8.02 (bs. -1H, NH); ^{13}C-NMR (60 MHz, $CDCl_3$); δ 26.17, 27.24, 30.59, 36.57 ($3CH_2$, NCH_3); 49.59 (NCH_2); 52.54, 59.07 (2CH); 66.46, 66.71, 66.86 (3OCH_2); 128.17, 128.26, 128.34, 128.55, 128.80, 135.27, 135.44, 135.79 (Ph-C); 171.25, 171.34, 172.01, 172.61, 173.05 (5C=O).

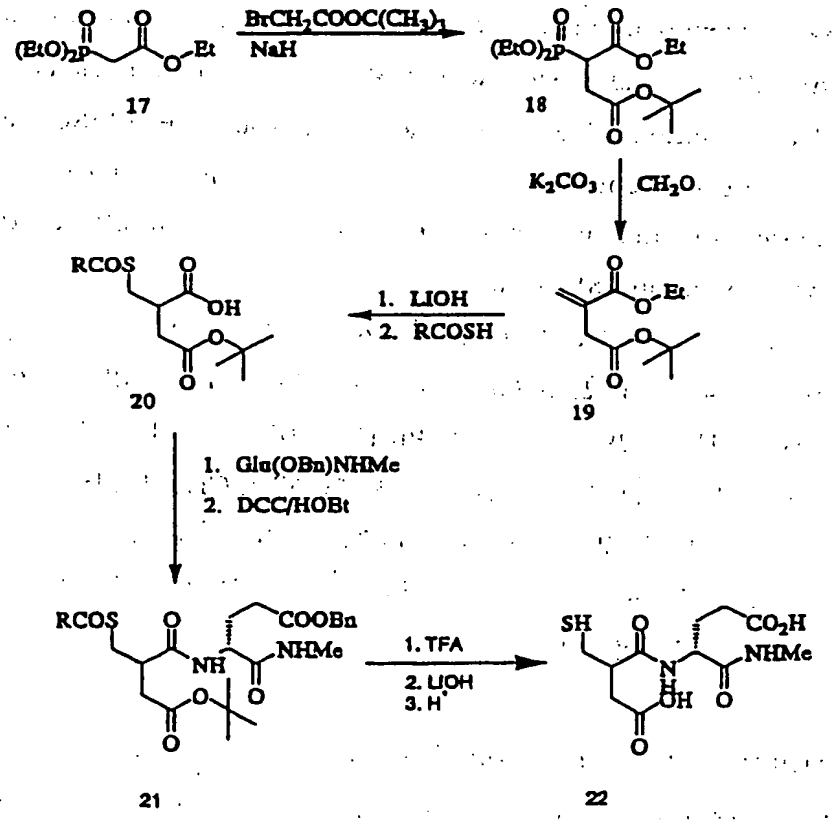
Synthesis Of N-Carboxymethyl-Asp-Glu-NMe (16): To a solution of benzyl-protected N-carboxymethyl dipeptide (15) (68 mg, 0.113 mmol) in methanol (5 ml) was added portionwise Pd/C powder (50 mg). The mixture was stirred under H_2 atmosphere (balloon pressure) at room temperature for

20 h. Catalyst was filtered of through a pad of celite and rinsed with methanol. The filtrate was concentrated and the crude solid was recrystallized from EtOAc/MeOH at -20°C to afford the product N-Carboxymethyl-Asp-Glu-NMe (16) (23 mg, 0.065 mmol) as a white solid in 58 % yield.

mp: 147-149 °C; NMR (360 MHz, DMSO-d6) δ 8.19 (d, J = 8.5 Hz, 1 H), 7.72 (m, 1 H), 4.20 (m, 1 H), 3.45-3.20 (m, 4 H), 2.63-2.44 (m, 5 H), 2.20 (m, 2 H), 1.93 (m, 1 H), 1.72 (m, 1 H).

6.1.5. Synthesis Of Mercapto Compound

The preferred method for synthesizing mercapto compound (22), also designated as FG-O74 (Example for Inhibitor C) is as follows:



(FG-074)

Alkylation of the diethyl phosphate (17) with tert-butyl bromacetate in the presence of NaH provided the phosphonate (18) in 90 % yield. Horner-Emmons reaction of (18) with formaldehyde using K_2CO_3, as a base resulted in the formation of the unsaturated ester (19). Saponification of (19) with LIOH and subsequent Michael addition of thiolacetic acid provided the acid (20). Coupling of (20) with Glu(OBn)NHMe using the DCC/HOBt method afforded the dipeptide (21) as a mixture of diastereomers by flash chromatography. Deprotection of (21) with TFA in CH_2Cl_2 provided the mono acid (22).

Synthesis 1-Ethyl-2-diethylphosphono-succinic acid 4-tert-butyl ester (18): To a suspension of NaH (0.48 g, 20 mmol) in dry THF (80 ml) was added dropwise a solution of triethyl phosphonoacetate (4.48 g, 20 mmol) in THF (20 ml) and after 3 h at room temperature tert.-butyl bromoacetate in THF (20 ml). The resulting suspension was stirred for 3 h at room temperature, water (50 ml) was added and the reaction mixture was acidified with 1 M HCl to pH 3. After addition of diethyl ether (70 ml), the organic was separated, washed with saturated NaCl solution, dried over $MgSO_4$ and the solvent was evaporated in vacuo, to give a colourless oil in 90 % yield.

Synthesis Of 1-Ethyl-2-methylene-succinic acid 4-tert.-butyl ester (19): A mixture of compound (18) (5.4 g, 16 mmol), K_2CO_3 (6.9 g, 50 mmol), and a 30% aqueous solution of formaldehyde (3.2 g, 100 mmol) was refluxed for 3 h. After cooling, the mixture was extracted with hexane, the organic layer was washed with water and brine and dried over $MgSO_4$. After filtration and evaporation in vacuo, an oily residue was obtained in 80% yield (2.7 g).

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.29 (t, j = 7 Hz, 3H, CH_2); 1.43 (s, 9H, CH_3); 3.24 (s, 2H, CH_2; 4.20 (q, J = Hz, 2H, OCH_2); 5.63, 6.28 (s, 2H, $=CH_2$).

Synthesis Of 2-Acetylsulfanylmethyl-succinic acid 4-tert.-butyl ester (20): The unsaturated ester (19) (2.3 g, 11 mmol) was dissolved in THF (50 ml) and a 0.2 M aqueous solution of LIOH (264 mg, 11 mmol) was added at 0°C. The mixture was allowed to come to room temperature and was stirred additional 3 h. The organic solvent was evaporated and the aqueous layer was extracted with diethyl ether (30 ml). The aqueous layer was acidified with 1 M HCl to pH 3 and extracted with EtOAc. The organic layer was washed with water and brine and dried over $MgSO_4$. After filtration and evaporation of the solvent, the acid was obtained as a white solid in 60% yield.

^{1}H-NMR (200 MHz, $CDCl_3$); δ 1.44 (s, 9H, CH_3); 3.26 (s, 2H, CH_2); 5.78, 6.42 (s, 2H, $=CH_2$); 10.01 (bs, 1H, COOH).

The acid (1.0 g, 5.4 mmol) was dissolved in $CHCl_3$ and thioacetic acid (1.45 g, 19 mmol) was added. The mixture was stirred at 60°C for 49 h. The solvent was evaporated in vacuo, and the compound (20) was obtained with a quantitative yield as a colourless oil.

^{1}H-NMR (200 MHz, $CDCl_3$); δ 1.44 (s, 9H, CH_3); 2.34 (s, 3H, CH_3); 2.45 - 2.75 (m, 2H, CH_2); 2.96 - 3.33 (m, 3H, CH_2, CH); 9.74 (bs, 1H, COOH).

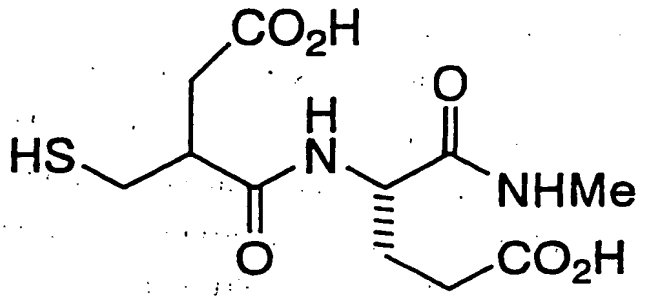
Synthesis Of 3-acetylthiomethyl-4-oxo-5-aza-6-(R)-methylcarbamoyl-8-benzyloxycarbonyl-octanoic Acid, tert-butyl Ester (21): To a solution of tert.-butoxycarbonyl amino ester (13) (700 mg, 2 mmol) in 10 ml CH_2Cl_2 was added 1 ml TFA and the reaction mixture was stirred for 1 h at room temperature under argon atmosphere. The excess acid was evaporated under vacuum, the residue was treated several times with diethyl ether (15 ml) and concentrated under reduced pressure, to give a colourless oil, which was used without further purification. The TFA-salt, acid (20) (525 mg, 2 mmol) HOBt (170 mg, 2 mmol) and NMM (202 mg, 2 mmol) were dissolved in CH_2Cl_2 (40 ml). A solution of DCC (412 mg, 2 mmol) in CH_2CH_2 (10 ml) was added and the reaction was stirred overnight at room temperature under argon atmosphere. The precipitate of dicyclohexylurea was removed by

filtration and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (30 ml), filtrated again and washed with 1 M $NaHCO_3$, 10% citric acid, and saturated NaCl solution. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, to give (21) as a colourless oil, which was purified by silica gel chromatography using EtOAc as eluant. The afforded yield was 72% (710 mg), as a mixture of diastereomers. ^1H-NMR (200 MHz, $CDCl_3$); δ 1.36, 1.39 (2s, 9H, CH_3); 1.87 - 3.2 (m, 14H, $4CH_3$, CH_3, NCH_3); 4.40 (m, 2H, CH); 5.08, 5.11 (2s, 2H, OCH_2); 6.43, 6.76, 7.07 (bs, -2H, NH); 6.76 (2d, 1H, NH); 7.31 (m, 15H, Ph-H).

Synthesis of 3-Thiomethyl-4-oxo-5-aza-6-(R)-methylcarbamoyl-8-carboxy-octanoic Acid (22) (FG 074): To a solution of tert-butoxycarbonyl amino ester 21 (495 mg, 1 mmol) in 15 mL CH_2Cl_2 was added TFA (1.5 mL) and the reaction mixture was stirred for 1h at room temperature under argon atmosphere. The solvent and excess acid were evaporated under vacuum, the residue was treated several times with diethyl ether (10 mL) and concentrated under reduced pressure, to give a colourless oil, which was purified by flash chromatography on silica gel (ethyl acetate/MeOH 10:1, containing 1% acetic acid) to yield the intermediate as a white solid 84% (370mg) yield.

To a solution of the intermediate (30 mg, 0.07 mmol) in 1 mL of (1.5/1) methanol/H_2O at room temperature was added $LiOH.H_2O$ (12 mg, 0.27 mmol). After stirring for 3 hours at room temperature, the reaction mixture was quenched with 0.5mL of 1 N HCl aqueous solution and extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated to get the thio compound 22 as a gummy solid (19 mg, 0.06 mmol) in 86% yield.

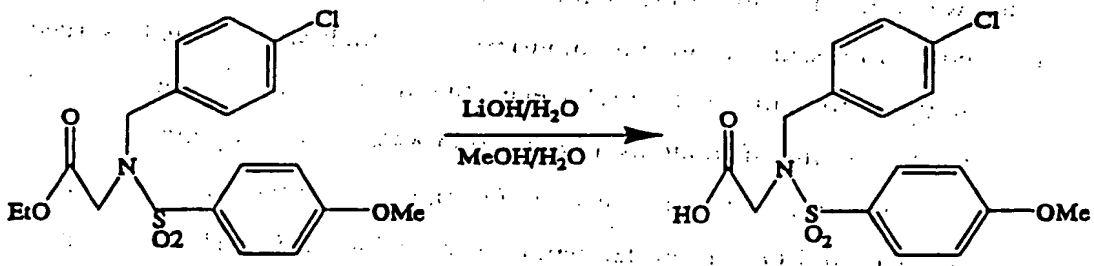
MS (ES) $(M + H)^+$: 307



22 (FG-074)

6.1.6. Synthesis Of 2-[[N-(4-methoxybenzenesulfonyl)-N-(4-chlorobenzyl)]amino]acetic Acid

The preferred method for synthesizing 2-[[N-(4-methoxybenzenesulfonyl)-N-(4-chlorobenzyl)]-amino]acetic acid (23), also designated as FG046, (Example for Inhibitor A) is as follows:



4 23 (FG 046)

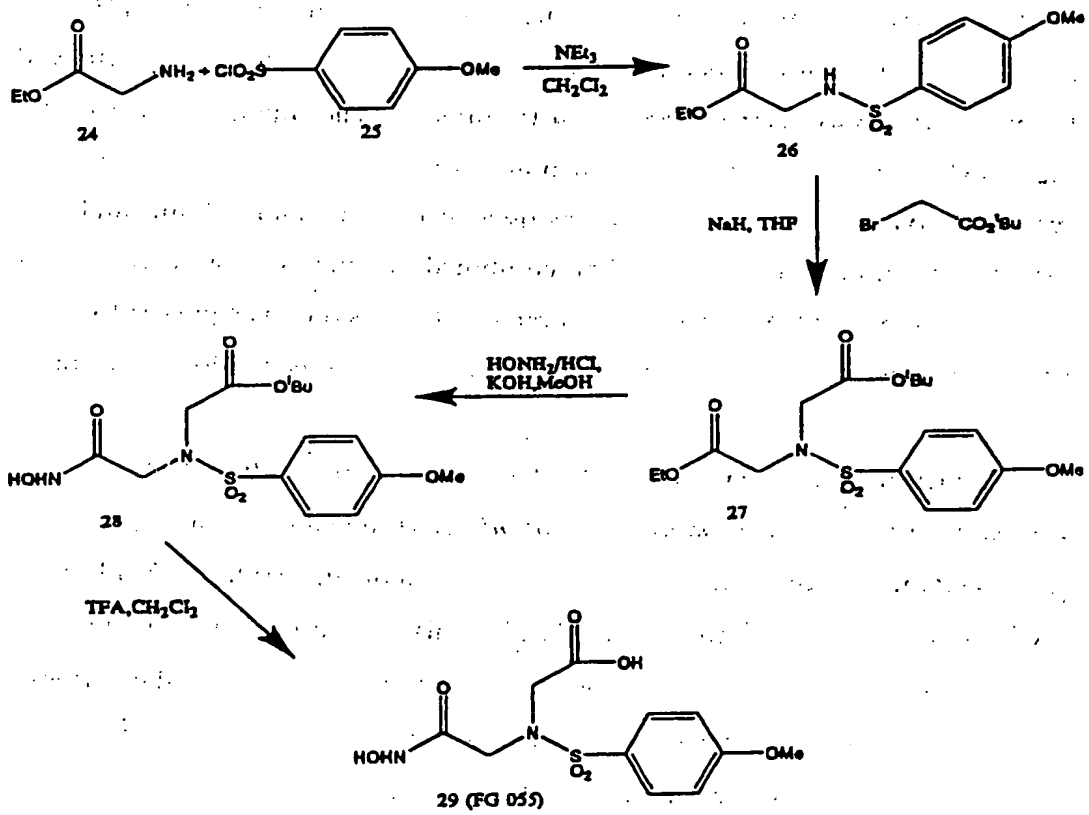
Synthesis Of 2-[[N-(4-methoxybenzenesulfonyl)-N-(4-chlorobenzyl)]amino]acetic Acid (23): To a suspension mixture of ethyl ester (4) (300 mg, 0.75 mmol) in 1.5:1 MeOH/H_2O (4ml) was added LiOH/H_2O. After stirring at room temperature for 5 h, the mixture was quenched with 1 N HCl solution (20 ml) and extracted with CH_2Cl_2 (2x20 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated.

The crude solid was recrystallized from hot ether to afford (23) as a white solid.

mp : 139.5-140°C; ^{1}H NMR (360 MHz, DMSO-d6) δ7.79 - 7.08 (m,4H,Ph); 4.37 (s,2 H,CH_2), 3.85 (s, 3H, OCH_3), 3.83 (s,2H, CH_2).

6.1.7. Synthesis Of N-hydroxy-2[[N'-(4-methoxybenzenesulfonyl)-N'(carboxymethyl)]amino]acetamide

The preferred method for synthesizing N-hydroxy-2[[N'-(4-methoxybenzenesulfonyl)-N'(carboxymethyl)]amino]acetamide (30), also designated as FG055, (Example for Inhibitor A) is as follows:



Synthesis Of Ethyl 2-[[N-(4-methoxybenzen-sulfonyl)]amino]acetate (26): To a mixture of glycine ethyl ester hydrochloride (24) (3.0 g, 21.5

mmol) and 4-methoxybenzenesulfonyl chloride (25) (4.4 g, 21.3 mmol) in anhydrous CH_2Cl_2 (60 ml) was added triethylamine (4.79 g, 47.3 mmol). After stirring at room temperature for 15 h, the reaction mixture was quenched with 1 N HCl (120 ml) and extracted with CH_2Cl_2 (2x100 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The crude solid was recrystallized from EtOAc/hexanes to give sulfonamide (26) as a white solid.

Synthesis Of Ethyl 2-[[N-(4-methoxybenzene-sulfonyl)-N-tert-butyloxycarbonylmethyl)]amino]acetate (27): To a slurry of sodium hydride (60% dispersed in mineral oil) (162 mg, 4.03 mmol) in anhydrous THF (10 ml) in an ice bath was added sulfonamide (26) (1.0 g, 3.66 mmol), followed by bromo tert-butylacetate (785 mg, 4.03 mmol). The mixture was stirred vigorously at 0°C for 30 min and then at rt for 15 h. After cooling the reaction mixture in an ice bath, it was quenched with water (25 ml) and extracted with ether (2x50 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography (2:1 hexanes:EtOAc) to give (27) as a colorless syrup.

Synthesis OfN-hydroxy-2[[N'-4-methoxybenzenesulfonyl)-N'-(tert-butyloxycarbonyl-methyl)]amino]acetamide (28): Separate solutions of hydroxylamine hydrochloride (377 mg, 5.43 mmol) in MeOH (2.7 ml) and of KOH (456 mg, 8.13 mmol) in MeOH (2.7 ml) were prepared at the boiling point, cooled to 40°C, and then the latter solution was added to the former. After cooling in an ice bath for 30 min, potassium chloride solid was filtered off. To the filtrate was added ethyl ester (27) (1.05 g, 2.71 mmol). After stirring at room temperature for 6 h, the reaction mixture was neutralized with 1 N HCl solution to pH 4, and partitioned between CH_2Cl_2(60 ml) and water (20 ml). The resulting two phases were separated and the aqueous layer was extracted with CH_2Cl_2 (60 ml). The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by silica

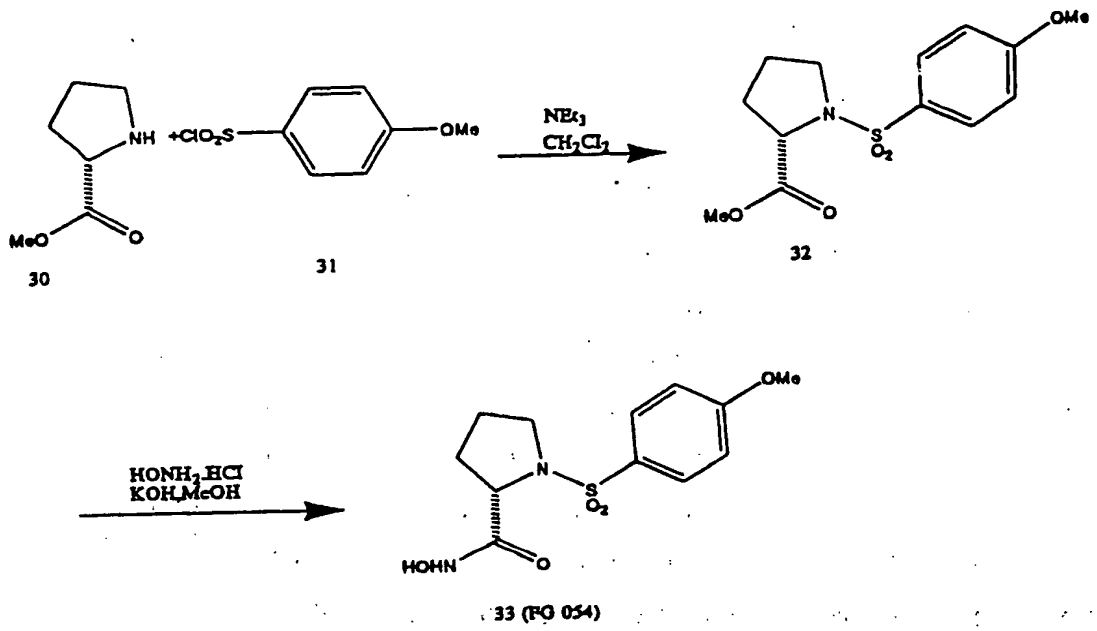
gel chromatography (11:1 CH_2Cl_2:MeOH) to give ester hydroxamate (28) also designated as FG-058, as a white solid.

Synthesis Of N-hydroxy-2[[N'-(4-methoxybenzenesulfonyl)-N'(carboxymethyl)]-amino]acetamide (29): A solution of tert-butyl ester hydroxamate (28) (520 mg, mmol) in 35% CF_3COOH/CH_2Cl_2 (9 ml) was stirred at 0°C for 10 min and then at room temperature for 1.5 h. The mixture was concentrated and dried *in vacuo*. The residue was triturated in EtOAc and the solid was collected which was recrystallized from EtOAc/MeOH/hexanes to give acid hydroxamate (29) as a white solid.

mp: 160-161 °C; MS (ES) $(M + H)^+$: 319; ^1H NMR (360 MHz, DMSO-d6) δ 12.08 (brs, 1 H, CO_2H), 10.69 (s, 1 H, OH), 8.96 (s, 1 H, NH), 7.76 (d, J = 8.7 Hz, 2 H, Ph), 7.09 (d, J = 8.7 Hz, 2 H, Ph), 4.01 (s, 2 H, CH_2), 3.84 (s, 3 H, OMe), 3.83 (s, 2 H, CH_2).

6.1.8. Synthesis Of N-(4-Methoxybenzene-sulfonyl)-L-proline Hydroxamate

The preferred method for synthesizing N-(4-Methoxybenzenesulfonyl)-L-proline hydroxamate, also designated as FG054, (Example for Inhibitor A) is as follows:



30 31 32

33 (FO 054)

Synthesis Of N-(4-Methoxybenzenesulfonyl)-L-Proline Methyl Ester

To a solution of L-proline methyl ester hydrochloride (30) (1.00 g, 6.03 mmol) and 4-methoxybenzenesulfonyl chloride (31) (1.19 g, 5.75 mmol) in anhydrous CH_2Cl_2 (17 ml) was added triethylamine (1.22 g, 12.06 mmol). After stirring at room temperature for 15 h, the reaction mixture was quenched with 1 N HCl (30 ml) in aqueous solution and extracted with EtOAc (2 x 100 ml). The combined EtOAc organic layers was washed with brine, dried over Mg_2SO_4, and concentrated to afford (32). This product was directly used for the next reaction without further purification.

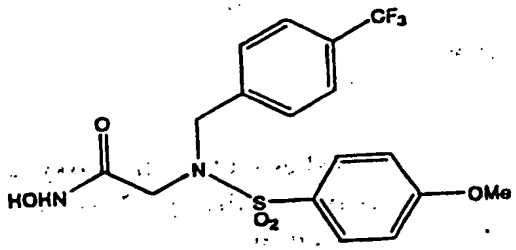
Synthesis Of N-(4-Methoxybenzenesulfonyl)-L-Proline Hydroxamate (33): Separate solutions of hydroxylamine hydrochloride (465 mg, 6.68 mmol) in MeOH (3.4 ml) and of KOH (561 mg, 10.0 mmol) in MeOH (3.4 ml) were prepared at the boiling point, cooled to 40°C, and then the latter solution was added to the former. After cooling in an ice bath for 30 min, potassium chloride solid was filtered off. To the filtrate was added ethyl ester (32) (1.0 g, 3.34 mmol). After stirring at room temperature for 15 h, the reaction mixture was treated with 1 N HCl (40 ml) solution, and extracted

with CH_2Cl_2/MeOH (10:1) The organic layer was washed with brine, dried over $MgSO_4$, and concentrated to get the crude product. The crude solid was recrystalized from hot MeOH/EtOAc to afford the hydroxamate (33) as a solid.

6.1.9. Synthesis Of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-trifluoromethylbenzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-trifluoromethylbenzyl)]amino]-acetamide (34), also designated as FG-066 (Example for Inhibitor A) is essentially as described for the synthesis of synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-chlorobenzyl)]amino]-acetamide, using as starting material 4-trifluoromethylbenzaldehyde instead of 4-chlorobenzaldehyde. *See*, Section 6.1.1., *supra*.

^1H NMR (360 MHz, DMSO-d6) δ 10.50 (s, 1 H, OH), 8.83 (s, 1 H, NH). 7.81 - 7.08 (m, 8 H, Ph), 4.45 (s, 2 H, CH_2CO), 3.86 (s, 3 H, OMe), 3.67 (s, 2 H, CH_2Ph).

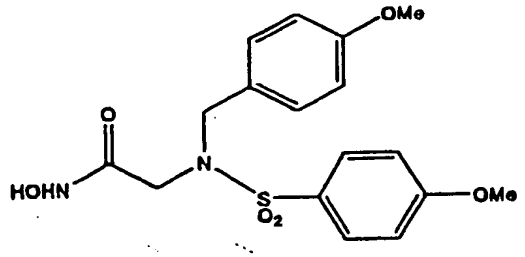


34 (FG-066)

6.1.10. Synthesis Of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-methoxybenzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-methoxybenzyl)]amino]-acetamide (35), also designated as FG-067 (Example for Inhibitor A) is essentially as described for the synthesis of synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N-(4-chlorobenzyl)]amino]-acetamide, using as starting material 4-methoxybenzaldehyde instead of 4-chlorobenzaldehyde. *See*, Section 6.1.1., *supra*.

^{1}H NMR (360 MHz, DMSO-d6) δ 10.42 (s, 1 H, OH), 8.79 (s, 1 H, NH). 7.81 - 6.86 (m, 8 H, Ph), 4.29 (s, 2 H, CH_2CO), 3.85 (s, 3 H, OMe), 3. 73 (s, 3 H, OMe), 3.28 (s, 2 H, CH_2Ph).



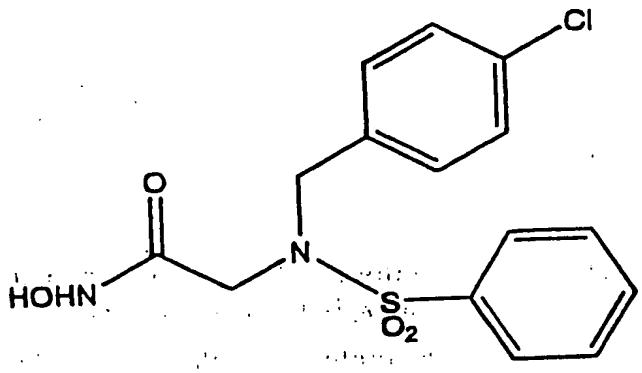
35 (FG-067)

6.1.11. Synthesis Of N-hydroxy-2-[[N'-(4-benzenesulfonyl)-N'-(4-chlorobenzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-benzenesulfonyl)-N'-(4-chlorobenzyl)]amino]-acetamide (36), also designated as FG-080 (Example for Inhibitor A) is essentially as described for the synthesis of synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(4-chlorobenzyl)]amino]-acetamide, using as

starting material benzenesulfonyl chloride instead 4-methoxybenzenesulfonyl chloride. *See*, Section 6.1.1., *supra*.

MS (ES) $(M + H)^+$: 355.

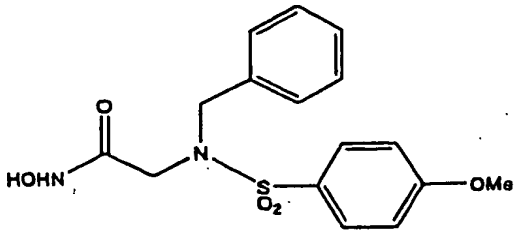


36 (FG-080)

6.1.12. Synthesis Of N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(benzyl)]amino]-acetamide

The preferred method for synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(benzyl)]amino]-acetamide (37), also designated as FG-061 (Example for Inhibitor A) is essentially as described for the synthesis of synthesizing N-hydroxy-2-[[N'-(4-methoxybenzenesulfonyl)-N'-(carboxymethyl)]amino]-acetamide, using as starting benzyl bromide instead of bromo tert-butylacetate. *See*, section 6.1.7., *supra*.

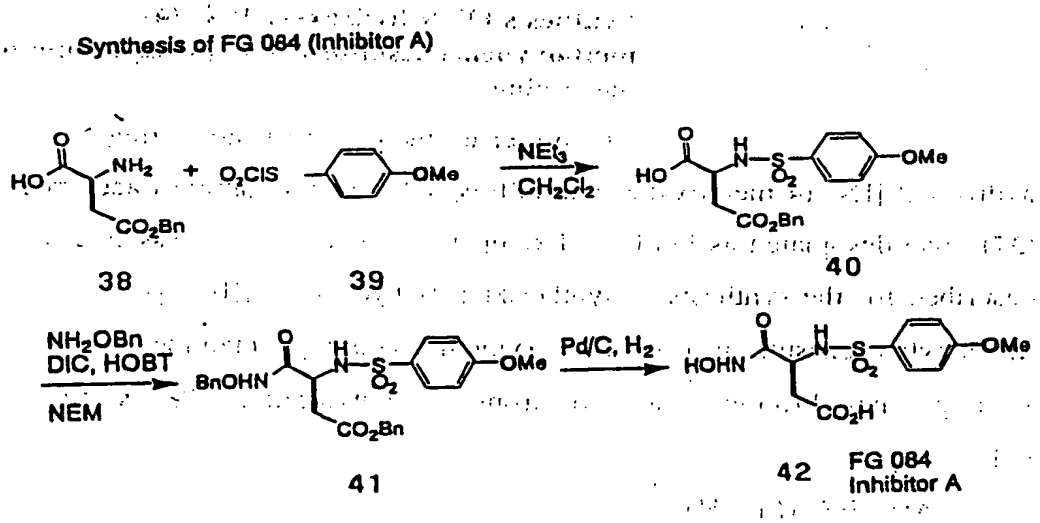
MS (ES) $(M - H)^-$:349



37 (FG-061)

6.1.13. Synthesis Of N-(4-Methoxybenzenesulfonyl)-β-benzyl-(L)-Aspartic Acid

The preferred method for synthesizing N-(4-Methoxybenzenesulfonyl)-b-benzyl-(L)-Aspartic Acid (42), also designated as FG084, (Example for Inhibitor A) is as follows:



N-(4-Methoxybenzenesulfonyl)-β-benzyl-(L)-aspartic Acid (40). To a suspension mixture of b-benzyl-(L)-aspartic acid HCl salt (38) (2.00 g, 8.96 mmol) and p-methoxybenzenesulfonyl chloride (39) (1.76 g, 8.53 mmol) in anhydrous CH_2Cl_2 was added triethylamine (1.81 g, 17.91 mmol) at room

temperature. After stirring for 15 hrs the reaction mixture was quenched with 1 N HCl (60 ml), and extracted with CH_2Cl_2 (3 x 50 ml). The combined organic layers was washed with brine, dried over $MgSO_4$, and concentrated to give N-(4- methoxybenzenesulfonyl)-b-benzyl-(L)-aspartic acid (3.14 g, 7.99 mmol, 94% yield) as a gummy product.

N-Benzyloxy-N'-(4-Methoxybenzenesulfonyl)-β-benzyl-(L)-aspartic amide (41). To a mixture of N-(4-methoxybenzenesulfonyl)-b-benzyl-(L)-aspartic acid (300 mg, 0.76 mmol) and O- benzylhydroxylamine/HCl in an anhydride solution of (7/3) THF/DMF (10 ml) was added N-hydroxybenzotriazole (HOBT) (103 mg, 0.76 mmol), N-ethylmorpholine (204 mg, 1.68 mmol) and then diisopropylcarbodiimide (106 mg, 0.84 mmol) at room temperature. After stirring over the weekend (2.5 days), the reaction mixture was diluted with (1/1) hexanes/EtOAc (40 ml), washed successively with 1 N HCl (2 x 20 ml), saturated NaHCO3 aqueous solution (2 x 20 mL) and brine. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel flash chromatography ((1/1) EtOAc/hexanes) to get N-benzyloxy-NŌ-(4-methoxybenzenesulfonyl)- b-benzyl-(L)-aspartic amide (114 mg, 0.22 mmol, 30% yield) as a white solid.

mp: 128 - 129 .C; MS (ES) $(M + H)^+$: 499

N-Hydroxy-N'-(4-Methoxybenzenesulfonyl)-(L)-aspartic amide (42). A mixture of N-benzyloxy-NŌ-(4-methoxybenzenesulfonyl)-b-benzyl-(L)-aspartic amide (102 mg, 0.20 mmol) and 10% Pd/C (43 mg) in methanol (7 ml) was stirred vigorously in a hydrogen atmosphere (balloon pressure) for 20 hrs. The catalyst was filtered off through a pad of celite and the filtrate was concentrated. The residue was lyophilized from water to get N-hydroxy-NŌ-(4-methoxybenzenesulfonyl)-(L)-aspartic amide (50 mg, 0.16 mmol, 77% yield) as a hygroscopic fluffy powder.

1H NMR (360 MHz, DMSO-d6) δ 10.60 (s, 1 H), 8.78 (s, 1 H), 7.87 (d, J = 8.2 Hz, 1 H), 3.93 (m, 1 H), 3.82 (s, 3 H), 2.50 (m, 1 H), 2.21 (dd, J = 16.0, 6.5 Hz, 1 H).

6.2. Example 2: C-Proteinase Assays

6.2.1. *In Vitro* Assay For The Determination Of C-Proteinase Activity And The IC_{50} Of Inhibitors

The following assay may be used to determine the level of activity and effect of the different compounds of the present invention on C-proteinase activity.

About 125 μg radiolabeled (^{14}C) procollagen is added to 10 units/ml of chicken C-proteinase in a solution of 0.1 M Tris-HCl, 0.1 M NaCl, 0.02% Brij-35, and 5 mM $CaCl_2$ in a total volume of 10 μl. The reaction is allowed to proceed for 15 minutes at 35° C and is stopped with one-half volume of 3x stop/loading buffer (30 mM EDTA, 30% glycerol, 6% SDS, 0.006% Bromophenol-blue). Subsequently, the samples are heated to 100°C for 4 minutes, and resolved by SDS-PAGE (Novex) using 6% polyacryleamide gels. The protein bands are detected by autoradiography. The amount of enzyme activity is based on the disappearance of the band corresponding to uncleaved procollagen.

The IC_{50} of inhibitors can be determined by plotting the % activity versus inhibitor concentration and estimating the inhibitor concentration which results in 50% activity.

The IC_{50} value of the inhibitors which have been tested is shown in TABLE I.

TABLE I:
IC_{50} Of Various Identified C-Proteinase Inhibitors.

Inhibitor	Generic Group	IC_{50}
FG-047	A	50μM
FG-061	A	100μM
FG-053	A	100μM
FG-052[1]	B	125μM
FG-066	A	150μM
FG-067	A	150μM
FG-086 (cbz-Pro-Leu-Gly-hydroxamate[2])	A	200μM
FG-087 (Ac-PYYG-hydroxamate)	A	335μM
FG-088 (Actinonin[3])	B	350μM
FG-054	A	400μM
FG-057	D	1500μM
FG-058[4]	A	2100μM
FG-055	A	2600μM
FG-051[5]	C	>> 100μM
FG-046	A	>> 1000μM

[1] commercially available from Peptides International (IHN-3850-PI).
[2] commercially available from Sigma (C-8537).
[3] commercially available from Sigma (A-6671).
[4] intermediate compound (28), see, section 6.1.7
[5] commercially available from Peptides International (ISN-3835-PI)

6.2.2. *In Vitro* ELISA Assay For The Determination Of C-Proteinase Activity And The IC_{50} Of Inhibitors

The IC_{50} value of the inhibitors can also be determined by a filtration ELISA assay. In this assay about 25 ng of unlabeled human procollagen I were incubated with the C-Proteinase as, *see*, section 6.2.1 but for one hour. The reaction was stopped with the addition of 40 μl precipitation buffer (0.5 X Reaction buffer, 0.1 mg/ml chicken collagen II, 10 μg/ml BSA, 7.5 mM EDTA). 25 μl of 75% ethanol was added and the reactions were mixed and incubated on ice for one hour to precipitate the procollagen. The soluble c-propeptide was separated from the precipitated collagen by filtering through a Millipore multiscreen-HV 0.45 μm hydrophilic plate using a Millipore multiscreen vacuum manifold. 20 μl of the filtrate was

removed and the amount of cleaved c-propeptide was determined by using the procollagen type I C-peptide (PIP) EIA kit from Takara Biomedicals.

For inhibition of hBMP-1 about 20 ng radiolabeled (^{125}I) human procollagen I was added to 1 to 2 µl of 5 times concentrated recombinant hBMP-1 cell media (Kessler et al. (1996) Science 271:360) in reaction buffer in a total volume of 10 µl. The reaction was let to proceed for one hour at 35 °C and then stopped with one-half volume of 3x stop/loading buffer and analyzed on SDS-PAGE as above.

The IC_{50} of the inhibitors was determined by plotting the % activity versus inhibitor concentration and estimating the inhibitor concentration which gives 50 % activity. IC_{50} values are shown in TABLE II.

TABLE II:
IC_{50} Of Various Identified C-Proteinase Inhibitors As Determined by ELISA

Inhibitor	Generic Group	IC_{50} ELISA
FG-061	A	12µM
FG-047	A	13µM
FG-053	A	22µM
FG-067	A	37µM
FG-066	A	48µM

6.2.3. Tissue Culture Assay For The Determination Of C-Proteinase Activity And The IC_{50} Of Inhibitors

C-proteinase activity and the IC_{50} of inhibitors *in vivo* may be determined in tissue culture assays by measurement of the production of procollagen and mature collagen in conditioned medium before and after treatment with a compound. The ratio of collagen and procollagen will directly correlate to the cellular conversion of the precursor to the mature collagen product, and as such indicate the C-proteinase activity.

Alternatively, the media content of C-propeptide/cell may be determined, and compared for untreated cells and inhibitor-treated cells.

6.3. Animal Models For The Determination Of C-Proteinase Activity And The Efficacy Of Inhibitors

Several animal models which mimic clinical disorders related to unregulated or inappropriate collagen production are known in the art and may be employed to determine the *in vivo* efficacy of the compounds of the invention. These animal models include a wound chamber model in rats (Schilling *et al.*, 1959, *Surgery* 46:702-710), an estradiol stimulated uterus expansion model (Mandell *et al.*, 1982, *The Journal of Biological Chemistry* 257:5268-5273), and an induced angiogenesis model (Matrigel) (Passaniti *et al.*, 1992, *Laboratory Investigation* 67:519-528). Further animal models include clinical disorder models like liver fibrosis models (Tsukamoto *et al.*, 1990, *Seminar in Liver Disease* 10:56-65; Kock-Weser, 1952, *Laboratory Investigation* 1:324-331; Marrione, 1949, *American Journal of Pathology* 25:273-285; Tams, 1957, *American Journal of Pathology* 33:13-27; Wahl *et al.*, 1986, *Journal of Experimental Medicine* 163:884-902), a pulmonary fibrosis model (Kelly *et al.*, 1980, *Journal of Laboratory Clinical Medicine* 96:954-964), arterial restenosis models (Jackson, 1994, *Trends of Cardiovascular Medicine* 4:122-130; Clowes *et al.*, 1983, *Laboratory Investigation* 49:327-333), a kidney fibrosis model (Yamamoto *et al.*, 1987, *Kidney International* 32:514-525), a tendon repairing model (Franklin *et al.*, 1986, *The Journal of Laboratory and Clinical Medicine* 108:103-108), a tumor growth model (Kiohs, *et al.*, 1985, *JNCL* 75:353-359), a trabeculectomy model (Lahery *et al.*, 1989, *Journal of Ocular Pharmacology* 5:155-179), and an abdominal adhesions model (Williams *et al.*, 1992, *Journal of Surgical Research* 52:65-70).

6.4. Example 4: Measurement Of Cytotoxicity

Potential inhibitors are studied in cytotoxicity assays in order to determine whether there is an effect on cell survival or proliferation. These assays may involve the use of rapidly proliferating or quiescent cells. A known number of cells is seeded and exposed for increasing periods of time to

a concentration range of potential inhibitors. Cell numbers are determined by cell counting or staining (*e.g.* Crystal Violet).

Cytotoxicity is evaluated as a function of cellular survival and cell proliferation. Cellular survival involves the use of quiescent cells and is determined by cell number (counting or staining). A decrease in cell number indicates cell loss, and thus an effect on cell survival. Cell proliferation involves the use of rapidly proliferating cells and is, as well, determined by cell number. Here a decrease in cell number relative to the untreated controls indicates an effect on cell proliferation.

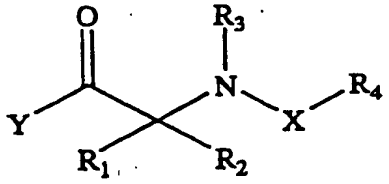
The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and any compounds and methods for the use thereof which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein are hereby incorporated by reference in their entirety.

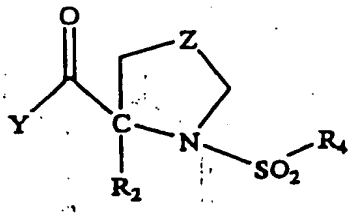
CLAIMS

What Is Claimed:

1. A compound having an inhibitory effect on C-proteinase, said compound having a formula selected from the group consisting of:



or:



and pharmaceutically acceptable salts thereof;

wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of aryl, heteroaryl, alkyl, aralkyl, heteroaralkyl, alkylamino, arylalkylamino;

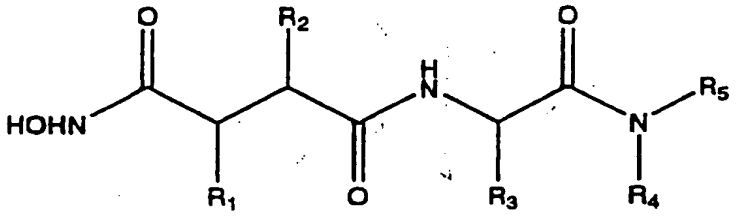
X is selected from the group consisting of SO_2, C=O;

Y is selected from the group consisting of OH, HOHN(hydroxylamine), H_2N, alkylamino;

Z is a direct bond; methylene, oxygen, sulfor, amino; and

n is 0 or 1.

2. A compound having an inhibitory effect on C-proteinase, said compound having a formula selected from the group consisting of:



and pharmaceutically acceptable salts thereof;

wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

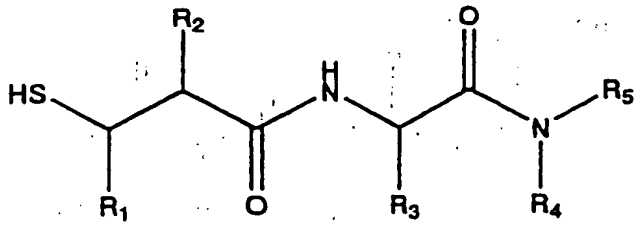
R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

3. A compound having an inhibitory effect on C-proteinase, said compound having a formula selected from the group consisting of:



and pharmaceutically acceptable salts thereof;

wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

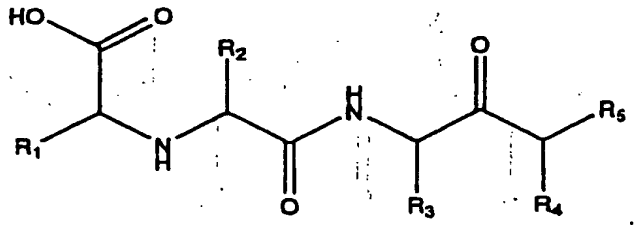
R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

4. A compound having an inhibitory effect on C-proteinase, said compound having a formula selected from the group consisting of:



and pharmaceutically acceptable salts thereof;

wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

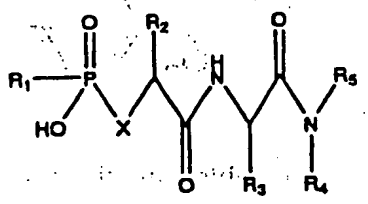
R_2 is selected from the group consisting of H, lower alkyl, mono-, or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

5. A compound having an inhibitory effect on C-proteinase, said compound having a formula selected from the group consisting of:



and pharmaceutically acceptable salts thereof;

wherein:

R_1 is selected from the group consisting of OH, alkoxyl, lower alkyl, alkylamino, peptide;

X is selected from the group consisting of N, C;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

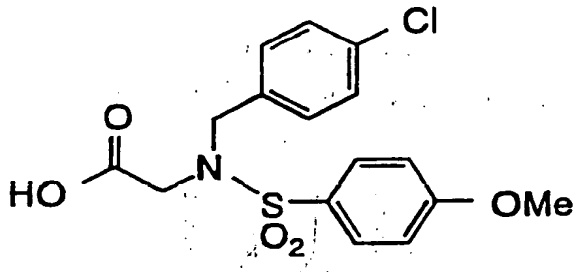
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino,

mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

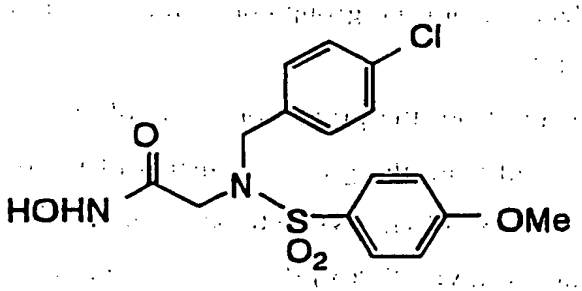
R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl.

6. The compound of Claim 1 wherein said compound has the formula:



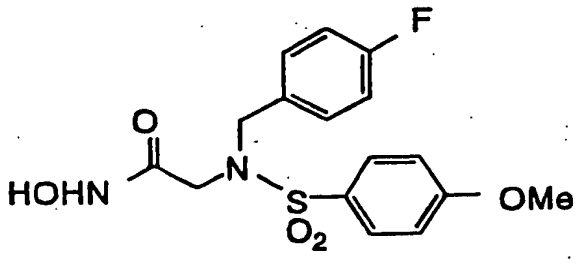
and pharmaceutically acceptable salts thereof.

7. The compound of Claim 1 wherein said compound has the formula:



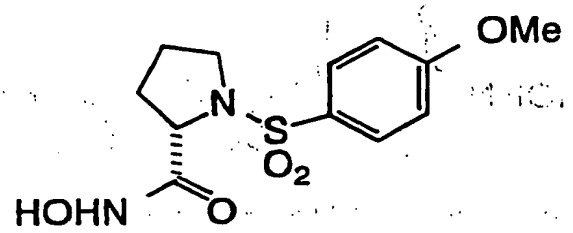
and pharmaceutically acceptable salts thereof.

8. The compound of Claim 1 wherein said compound has the formula:



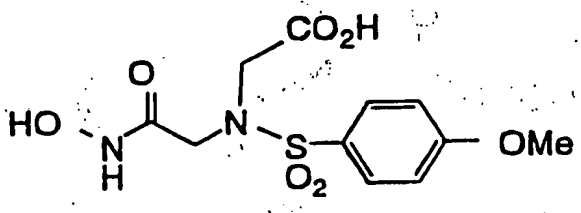
and pharmaceutically acceptable salts thereof.

9. The compound of Claim 1 wherein said compound has the formula:



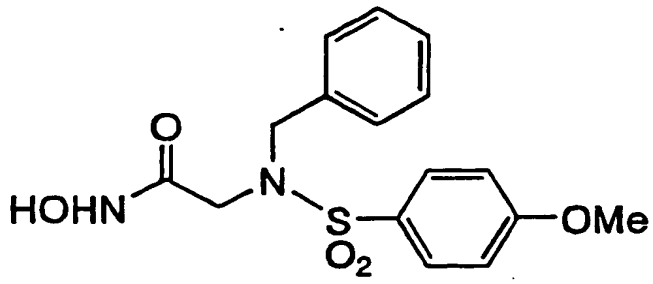
and pharmaceutically acceptable salts thereof.

10. The compound of Claim 1 wherein said compound has the formula:



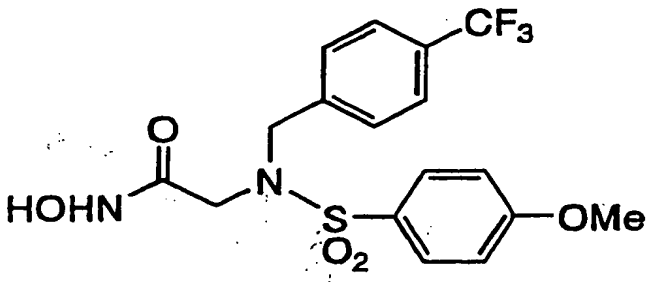
and pharmaceutically acceptable salts thereof.

11. The compound of Claim 1 wherein said compound has the formula:



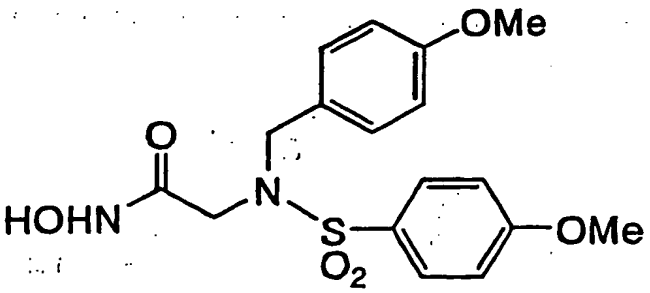
and pharmaceutically acceptable salts thereof.

12. The compound of Claim 1 wherein said compound has the formula:



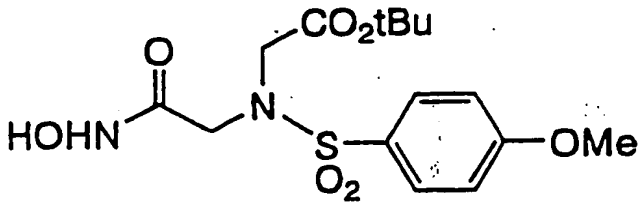
and pharmaceutically acceptable salts thereof.

13. The compound of Claim 1 wherein said compound has the formula:



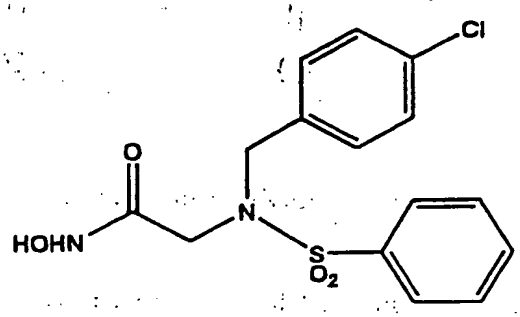
and pharmaceutically acceptable salts thereof.

14. The compound of Claim 1 wherein said compound has the formula:



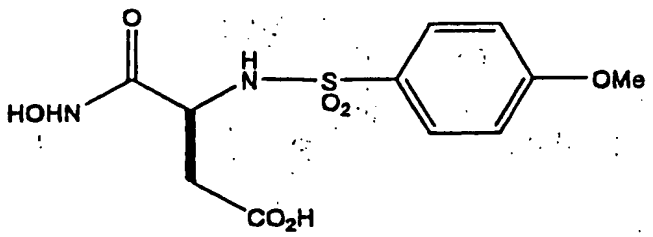
and pharmaceutically acceptable salts thereof.

15. The compound of Claim 1 wherein said compound has the formula:



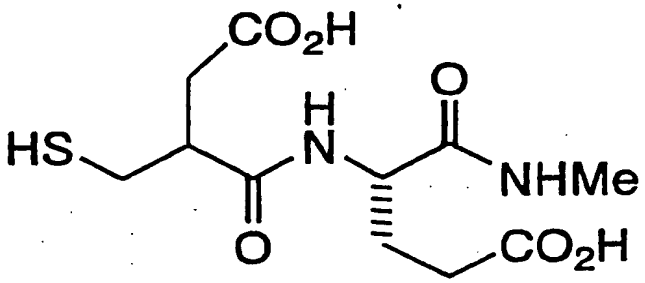
and pharmaceutically acceptable salts thereof;

16. The compound of Claim 1 wherein said compound has the formula:



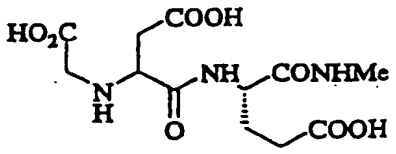
and pharmaceutically acceptable salts thereof;

17. The compound of Claim 3 wherein said compound has the formula:



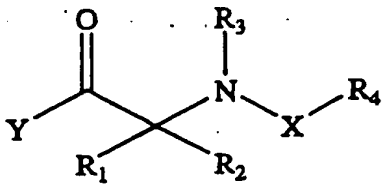
and pharmaceutically acceptable salts thereof.

18. The compound of Claim 3 wherein said compound has the formula:

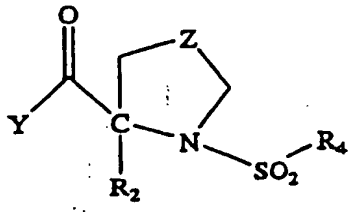


and pharmaceutically acceptable salts thereof.

19. A pharmaceutical composition comprising a compound selected from the group consisting of:



or:



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, halo substitituted aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of aryl, heteroaryl, alkyl, aralkyl, heteroaralkyl, alkylamino, arylalkylamino;

X is selected from the group consisting of SO_2, C=O;

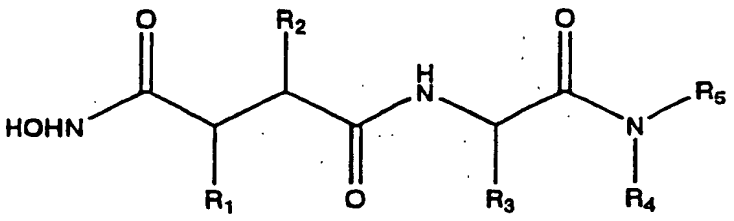
Y is selected from the group consisting of OH, HOHN(hydroxylamine), H_2N, alkylamino;

Z is a direct bond; methylene, oxygen, sulfor, amino; and

n is 0 or 1;

and a pharmaceutically acceptable carrier or excipient;

or:



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino,

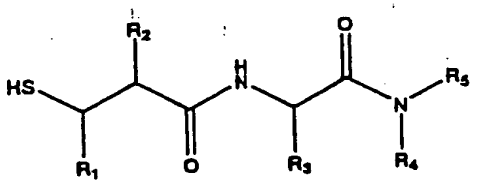
mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

and a pharmaceutically acceptable carrier or excipient;

or:



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono-, or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono-, or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

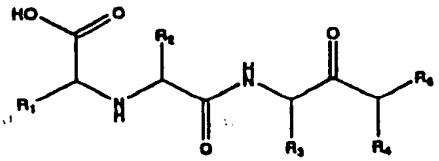
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl; (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

and a pharmaceutically acceptable carrier or excipient;

or:



wherein:

R_1 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

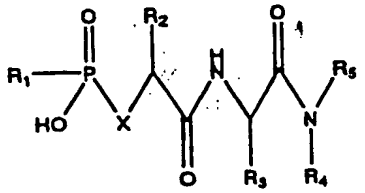
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

and a pharmaceutically acceptable carrier or excipient;

or:



wherein:

R_1 is selected from the group consisting of OH, alkoxyl, lower alkyl, alkylamino, peptide;

X is selected from the group consisting of N, C;

R_2 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

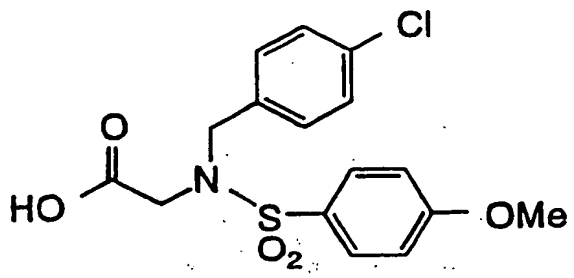
R_3 is selected from the group consisting of H, lower alkyl, mono- or poly-haloalkyl, carboxyalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, biaryl, biarylalkyl, hydroxyalkyl, alkyoxyalkyl, acyloxyalkyl, mercaptoalkyl, (amino, mono- or dialkylamino)alkyl, acylaminoalkyl, cycloalkyl, heterocycloalkyl, cycloalkylalkyl, heterocycloalkylalkyl, alkyl-(thio, sulfinyl or sulfonyl)-alkyl;

R_4 is selected from the group consisting of H, lower alkyl; and

R_5 is selected from the group consisting of H, lower alkyl, carboxyalkyl, (mono- or dialkylamino)alkyl, alkyl-(thio, sufinyl or sulfonyl)alkyl, alkoyalkylacylalkyl;

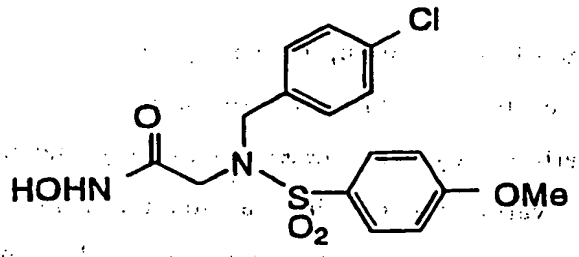
and a pharmaceutically acceptable carrier or excipient.

20. A pharmaceutical composition comprising a compound selected from the group consisting of:



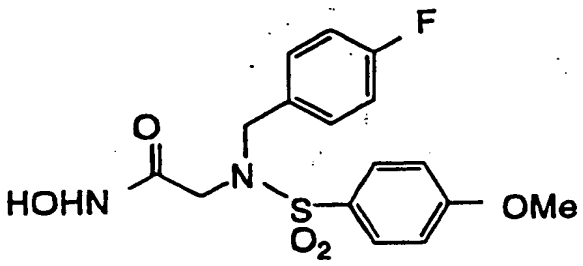
and a pharmaceutically acceptable carrier or excipient;

or:



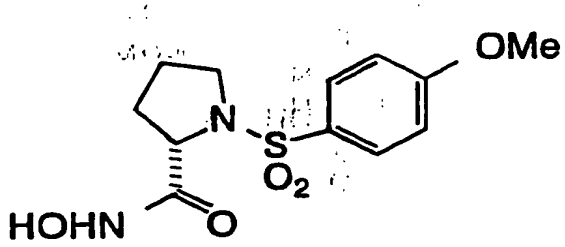
and a pharmaceutically acceptable carrier or excipient;

or:



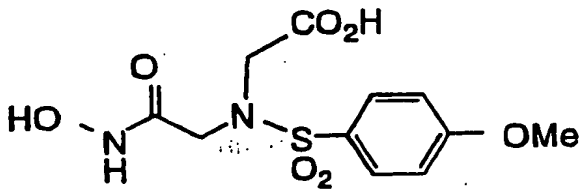
and a pharmaceutically acceptable carrier or excipient;

or:



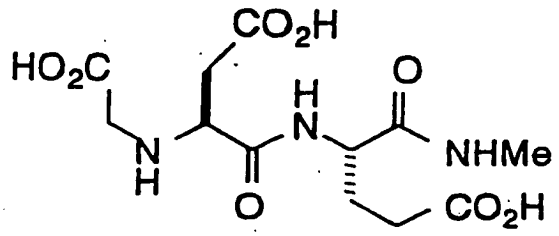
and a pharmaceutically acceptable carrier or excipient;

or:



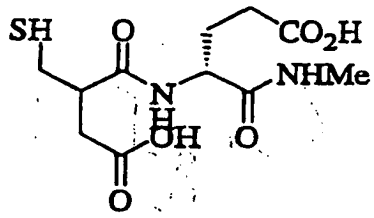
and a pharmaceutically acceptable carrier or excipient;

or:



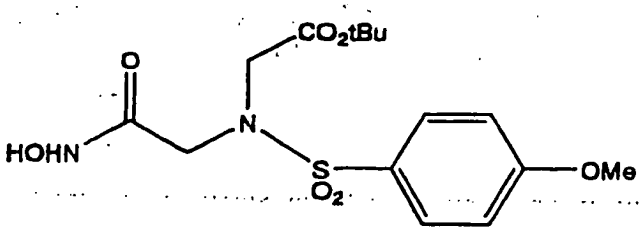
and a pharmaceutically acceptable carrier or excipient;

or:



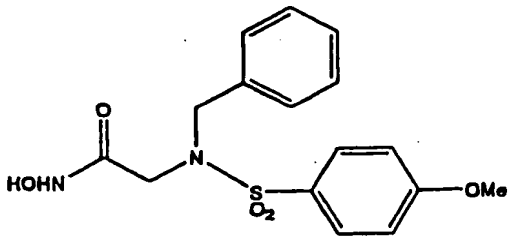
and a pharmaceutically acceptable carrier or excipient;

or:



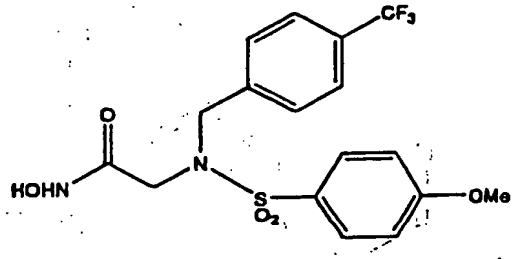
and a pharmaceutically acceptable carrier or excipient;

or:



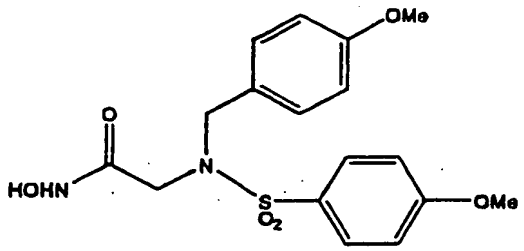
and a pharmaceutically acceptable carrier or excipient;

or:



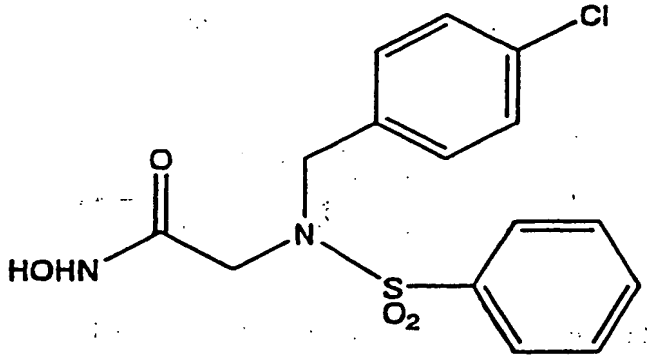
and a pharmaceutically acceptable carrier or excipient;

or:



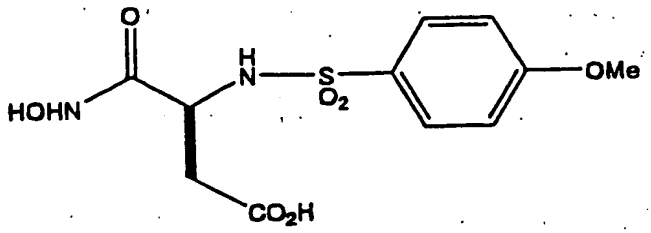
and a pharmaceutically acceptable carrier or excipient;

or:



and a pharmaceutically acceptable carrier or excipient;

or:



and a pharmaceutically acceptable carrier or excipient;

21. A method for treating diseases related to the inappropriate or unregulated production of collagen comprising administration of an effective amount of the composition according to Claim 20.

22. A method for treating diseases related to the inappropriate or unregulated production of collagen comprising administration of an effective amount of the composition according to Claim 21.

23. The method of Claim 22 wherein the fibrotic disorder is selected from the group consisting of hepatic cirrhosis and arthritis.

24. The method of Claim 23 wherein the fibrotic disorder is selected from the group consisting of hepatic cirrhosis and arthritis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12876

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/215, 31/38, 31/40, 31/66 31/165, 31/70, 31/34
US CL : 548/201, 215, 300.1, 542; 558/170; 564/192

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/201, 215, 300.1, 542; 558/170; 564/192

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CASLINK cluster, APS text search on c-proteinase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,237,057 A (BUECHLER et al.) 17 August 1993, see entire document.	1, 6-16, 19-24
Y	US 5,298,490 A (HEAVNER et al.) 29 March 1994, see entire document.	1, 6-16, 19-24
Y	US 5,393,902 A (COOPE et al.) 28 February 1995, see entire document.	1, 6-16, 19-24
Y	US 5,256,657 A (SINGH et al.) 26 October 1993, see entire document.	2, 19
Y	US 5,114,953 A (GALARDY et al.) 19 May 1992, see entire document.	2, 19
Y	US 4,599,361 A (DICKENS et al.) 08 July 1986, see entire document.	2, 19

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 NOVEMBER 1996	Date of mailing of the international search report 07 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAURA CROSS for Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12876

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,292,926 A (MORITA et al.) 08 March 1994, see entire document.	3, 17-19
Y	US 5,024,835 A (RAO et al.) 18 June 1991, see entire document.	3, 17-19
Y	US 5,276,147 A (THORNBACK et al.) 04 January 1994, see entire document.	3, 17-19
Y	US 5,443,815 A (DEAN et al.) 22 August 1995, see entire document.	4, 19
Y	US 5,149,794 A (YATVIN et al.) 22 September 1992, see entire document.	5, 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12876

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12876

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1, 6-16 and 19-24, drawn to compounds and compositions.
Group II, claim(s) 2 and 19, drawn to compounds and compositions.
Group III, claim(s) 3 and 17-19, drawn to compounds and compositions.
Group IV, claim(s), 4 and 19 drawn to compounds and compositions.
Group V, claim(s) 5 and 19, drawn to compounds and compositions.
The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the compounds lack a common inventive core. A -N-C(O)-CH2 group is the only common feature among all the claims.